Environmental and Experimental Botany 146 (2018) 13–26

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

# The dioecious *Populus tremula* displays interactive effects of temperature and ultraviolet-B along a natural gradient☆

Christian B. Strømme[a,*], Riitta Julkunen-Tiitto[c], Jorunn E. Olsen[b], Line Nybakken[a]

[a] Department of Ecology and Natural Resource Management, Faculty of Environmental Sciences and Natural Resource Management, CERAD, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway
[b] Department of Plant Sciences, Faculty of Biosciences, CERAD, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway
[c] Department of Biology, University of Eastern Finland, P.O. Box 111, FI-20101 Joensuu, Finland

ARTICLE INFO

*Keywords:*
Phenology
Growth
Phenolic compounds
Climate change
Ultraviolet
Dioecious

ABSTRACT

Observed and projected warming has provoked research interest on tree phenology in relation to climate. While both ultraviolet-B (UV-B) radiation and warming has been shown to affect growth and development in plants, the combined effects of the two environmental factors has been little studied. In addition, there is concern for environmental change yielding divergent responses between sexes in dioecious tree species. Here, we present a study of the dioecious *Populus tremula* grown along a natural temperature and UV-B gradient in Central Norway, reducing UV-B using specific screening filters. We tested for effects on growth, timing of terminal bud formation and bud break, carbon and nitrogen content and concentrations of phenolic compounds. Increased elevation had a negative effect on plant growth and promoted bud formation, with effects differing between plant sexes. UV-B attenuation delayed bud formation and enhanced growth of males at the highest elevation, counteracting the effect of low temperature. In addition, elevation and UV-B affected concentrations of different phenolics in stems and leaves. Our data show that interactive effects of warming and other climate factors like UV-B should be considered when predicting climate change effects in woody plants, and add to present evidence of sex-related responses to climate change in dioecious woody plants.

## 1. Introduction

The boreal forest accounts for a third of Earth's forest area, and is also the biome that exerts the largest biogeochemical influence on average global temperature (Snyder et al., 2004). During the latest decades, substantial warming has been observed at high latitudes (Serreze et al., 2000; Hartman et al., 2013), and observed changes for boreal tree species include increased growth (Jacoby & D'Arrigo 1995; Hember et al., 2012; Kauppi et al., 2014; Schaphoff et al., 2016), latitudinal and elevational range shifts (Kullman 2001; Zhu et al., 2012; Koven 2013; Monleon and Lintz 2015) and advanced spring phenology (Menzel et al., 2006; Bertin, 2008). In relation to phenology, there is evidence suggesting that further warming, which is predicted for the 21st century (IPCC, 2014), yields different responses across tree species (Roberts et al., 2015). It is well known that phenology in temperate and boreal tree species is driven by seasonal fluctuations of temperature and light (Olsen and Lee, 2011), but evidence from the rather few species that have been studied suggests that there is great interspecific variation in how species respond to day-length, temperature and light quality (Hänninen and Tanino, 2011). Furthermore, evidence from studies involving dioecious tree species show that females and males may respond differently to warming (Tognetti, 2012). Indeed, divergent responsiveness to environmental change between sexes have been shown in *Salix myrsinifolia* (Nybakken et al., 2012; Nybakken and Julkunen-Tiitto, 2013), *Salix arctica* (Dawson and Bliss, 1989) and *Populus cathayana* (Xu et al., 2008, 2010; Zhao et al., 2009, 2012; Jiang et al., 2015; Zhang et al., 2017). If such effects yield different performance between females and males, gender balance may play an important role for population dynamics under environmental change.

In addition to warming, boreal tree species are facing changes in precipitation, freeze/thaw cycles, $CO_2$ concentration and ultraviolet radiation. Some studies have revealed interactive effects in response to warming and supplemental Ultraviolet B (UV-B) radiation (Nybakken et al., 2012; Randriamanana et al., 2015; Strømme et al., 2015), and UV-B has also been shown to interact with drought (Ren et al., 2007) and soil nutrient availability (Ren et al., 2010; Feng et al., 2014). As climate change is projected to affect the thickness of the ozone column, it will also affect the levels of UV-B radiation received by Earth's surface (McKenzie et al., 2011; Williamson et al., 2014). Even though the successful implementation of the Montreal Protocol reduced the

☆ This article is part of a special issue entitled Sexual dimorphism in response to stress published at the journal Environmental and Experimental Botany 146C.

* Corresponding author.
*E-mail address:* christian.stromme@nmbu.no (C.B. Strømme).

http://dx.doi.org/10.1016/j.envexpbot.2017.09.013
Received 31 March 2017; Received in revised form 26 September 2017; Accepted 29 September 2017
Available online 27 October 2017
0098-8472/ 

depletion of stratospheric ozone, substantial variability of the ozone column thickness is expected due to warming effects on circulation patterns. Indeed, such an effect was observed during the record ozone depletion event over the Arctic in 2011 (Manney et al., 2011), yielding higher than normal levels of UV-B radiation from the North Pole to Southern Scandinavia. Considering available evidence on the physiological responses of species to UV-B radiation, such events are likely to affect ecosystem functioning (see Bornman et al. (2015) for a review). Even so, there are substantial knowledge gaps in relation to interactions between UV-B and other environmental factors undergoing rapid change. Moreover, several studies of UV-B effects on trees have employed supplemental UV-B from lamps either in semi- controlled or in field conditions (Xu et al., 2010; Nybakken et al., 2012; Strømme et al., 2015). To a smaller extent, effects have also been tested under the actual UV-B levels that plants are subjected to in nature (Caldwell et al., 2003), employing UV-B specific attenuation filters (Ballaré et al., 1996, 2001; Huiskes et al., 2001; Singh and Singh, 2014).

Plants are known to respond to UV-B exposure through variations in the synthesis of phenolic compounds, which may in turn affect stress tolerance as well as resistance to herbivory and pathogens (Ballaré et al., 2011). Increased phenolics synthesis under UV-B has been shown for boreal tree species such as *Betula pendula* (Lavola et al., 1997), *S. myrsinifolia* (Tegelberg and Julkunen-Tiitto, 2001) and *Populus tremula* (Lavola et al., 2013; Randriamanana et al., 2015). On the other hand, UV-B has been shown to decrease growth parameters in some woody species (Bassman and Robberecht, 2001; Tegelberg et al., 2003; Ren et al., 2007, 2010; Feng et al., 2014; Terfa et al., 2014), while other reports find no such effect (Tegelberg et al., 2001, 2003; Kotilainen et al., 2009; Morales et al., 2010; Lavola et al., 2013). Even so, growth reduction under UV-B is considered lower for woody plants as compared to herbaceous plants (Caldwell et al., 2003; Li et al., 2010).

UV-B may affect tree growth through environmental signalling pathways, and such effects have gained increased attention following the characterisation of a UV-B specific photoreceptor, known as UV-B resistance locus 8 (UVR8), in *Arabidopsis thaliana* (Jenkins, 2009). There is considerable evidence showing that UV-B is a signal that regulates plant growth and morphology through action on metabolism of hormones such as auxin and gibberellin (Rozema et al., 1997; Jansen, 2002; Rizzini et al., 2011; Jansen and Bornman, 2012; Hayes et al., 2014; Roro et al., 2017). UV-B was also shown to be involved in photoperiodic sensing in *A. thaliana* (Fehér et al., 2011). UV-B can thus act as an environmental cue in relation to phenology in plants. For a range of boreal tree species, light quality has been shown to be involved in phenological transitions (Olsen and Lee, 2011), namely far red (FR) (Junttila and Kaurin, 1985; Olsen et al., 1997a; Clapham et al., 1998; Tsegay et al., 2005; Mølmann et al., 2006) and blue light (Opseth et al., 2016). Thus, possible UV-B effects on boreal tree species should be investigated using an integrated approach that considers phenological transitions, phenolic synthesis and growth.

Moreover, effects of UV-B radiation on boreal trees should be tested in combination with warming. A survey of previous literature suggests that warming may both delay and accelerate phenological events in autumn, namely growth cessation and bud formation (Hänninen and Tanino, 2011). Day-length was early recognised to govern autumnal growth cessation and bud formation in trees (Wareing, 1956; Nitsch, 1957; Weiser, 1970). It has also been shown that temperature also affect these processes (Kalcsits et al., 2009; Tanino et al., 2010; Hänninen and Tanino, 2011; Rohde et al., 2011a; Strømme et al., 2015, 2017). As such, warming may interact with day-length (Ruttink et al., 2007; Søgaard et al., 2008; Tanino et al., 2010; Olsen et al., 2014), yielding a different phenological response to the day-length signal.

The dioecious Eurasian aspen (*Populus tremula*) has a wide distribution across the Eurasian continent, and is host to numerous species of birds, mammals, invertebrates, lichens and fungi. Due to its ease of propagation and wide environmental tolerance, it is widely used in plant physiological research. In a recent field study on Eurasian aspen grown in a modulated temperature and UV-B enhancement system, we found interacting effects of temperature and UV-B radiation on autumnal bud formation and bud break (Strømme et al., 2015). Autumn warming delayed bud formation, while enhanced UV-B levels in the same period had an opposite effect. Furthermore, we found that male plants were more responsive to both treatments, which also yielded advanced bud break the following spring.

The aim of this study was to simulate dual-factor climate change for female and male plants of the dioecious Eurasian aspen by employing a natural temperature and UV-B gradient. For this purpose, we established three experimental locations at different elevations in a valley slope in Central Norway. With increasing elevation, we obtained decreasing temperature and increasing UV-B radiation. We conducted two field experiments at the same locations over two consecutive years, and during the second year we employed attenuation filters mounted over plots to obtain reduced UV-B levels on subsets of new plant materials. Using the same clones as in Strømme et al. (2015) in order to account for possible responses related to genotypes, we hypothesised that 1) higher temperature at low elevation would lead to a more pronounced growth of plants, while also yielding a longer growing season due to delayed bud formation in autumn as well as earlier bud break in spring. We further hypothesised that 2) delayed bud formation for plants at low elevation would be accompanied by higher leaf nitrogen content due to delayed relocation from leaf to stem. Based on Strømme et al. (2015), we also hypothesised that 3) attenuating UV-B radiation would delay autumnal bud formation, and that elevated temperature and UV-B would yield stronger responses in males. Based on available literature, we hypothesised that 4) UV-B attenuation would yield reduced synthesis of polyphenols in leaves, particularly flavonoids and thereby higher growth rates due to reduced allocation to defence.

## 2. Materials and methods

### 2.1. Plant material

Plants used in the field experiments originated from six female and six male aspens located in Southern and Eastern Finland (62°54′-„ 60°43′ N, 24°27′ − 29°41′ E). For a thorough description of sampling locations and micropropagation of individuals see Strømme et al. (2015). Plants were potted on 4 June 2013 and on 10 June 2014 using 70% non-fertilised peat and 30% vermiculite. Prior to planting in the field, plants were kept in growth chambers under 237 ± 5 $\mu mol\ m^{-2}\ s^{-1}$ at 400–700 nm and a red: far-red (R:FR) ratio of 1.6 ± 0.1 provided by 400W Philips MASTER HPI-T Plus metal halide lamps (Royal Philips, Amsterdam, Holland) and incandescent light bulbs (60 W, Osram, Munich, Germany). Photosynthetic active radiation (PAR) was measured using a LI-250 Light Meter with an attached Quantum Sensor (LI-COR, Lincoln, Nebraska, USA), while R:FR ratio was measured using a Sky 100 radiometer with an attached 660/730 nm sensor (Skye Instruments, Llandrindod Wells, UK). The first days after potting, the plants were kept under a semi-translucent plastic sheet, which was gradually removed. This provided a gradual climatic shift in terms of irradiance, temperature and relative air humidity (RH). In the growth chambers, temperature and RH were 20 °C and 75%, respectively, and progressively lowered to 16 °C and 65% over seven days in 2013 and four days in 2014 to allow acclimation to lower temperature and RH. Plants were planted in the outdoor experimental locations on 4 July in 2013 and on 24 June in 2014. Later planting in 2013 was due to cooler spring/early summer conditions this year as compared to 2014.

### 2.2. Experimental set up

We stablished experiments at three different elevations in Fåvang, Central Norway (61°27′ N, 10°11′ E) along the eastern side of the Gudbrandsdalen valley. Each location was a pasture selected with the aim of having three sites at different elevations along a natural gradient

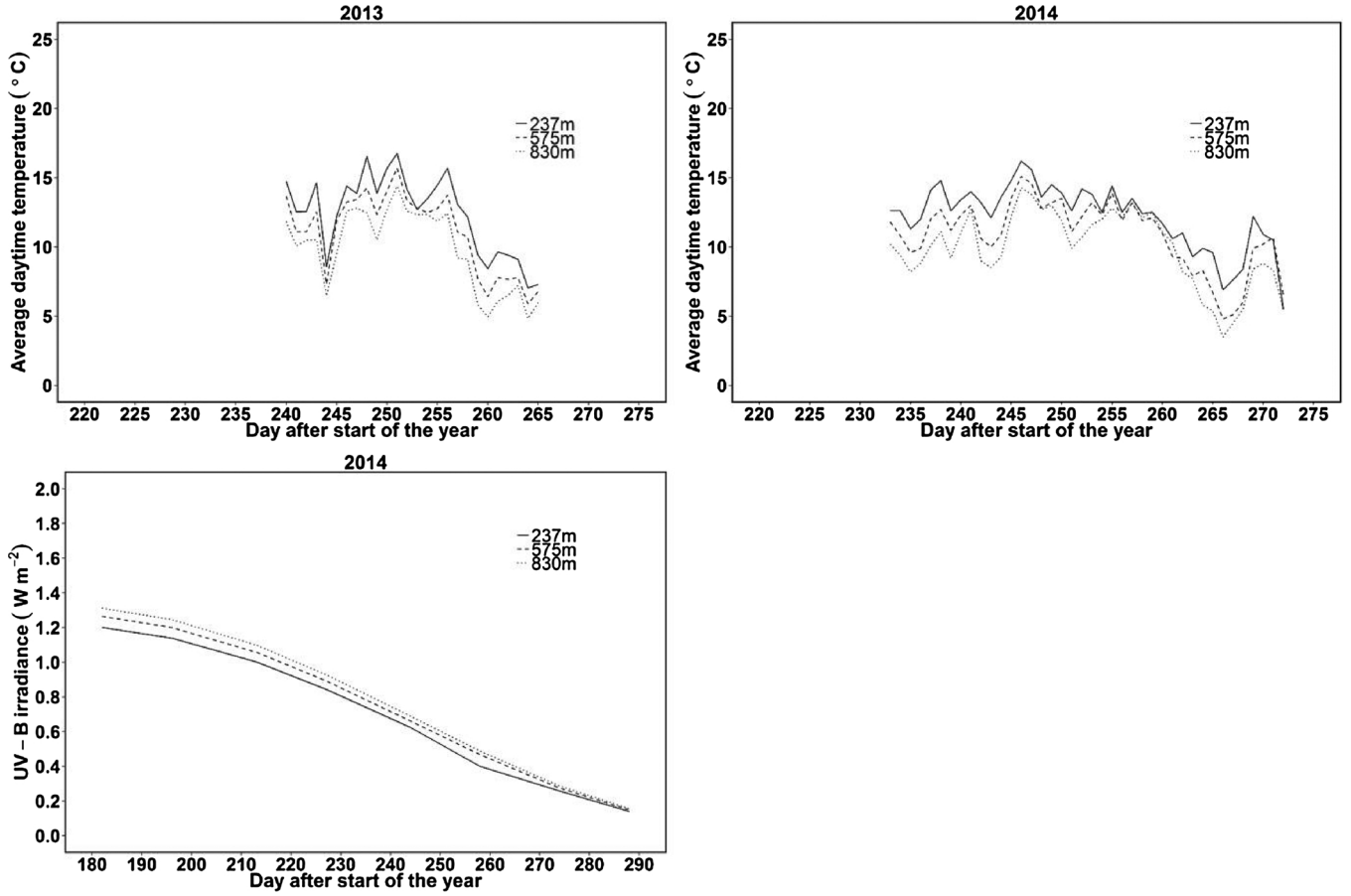


**Fig. 1.** Daily average temperatures for different elevations during autumn 2013 (top left) and 2014 (top right) and calculated UV-B irradiance (bottom) for the different locations using the Quick TUV Calculator of the NCAR Earth System Laboratory.

while keeping irradiance and topography as similar as possible. Selected sites were located at 237, 575 and 830 m a.s.l. along a 3.8 km long west-east axis. Large herbivores were excluded from each location using a 2.5 m high metal wire fence. In 2013, five 180 × 120 cm plots per location were set-up by removing the uppermost 10 cm of soil, in addition to rocks in the exposed sublayer. The total removed material was replaced by 10 cm of FLORALUX peat compost (pre-limed and pre-fertilised) (Nittedal Torvindustri, Arneberg, Norway) in order to reduce differences in soil conditions between sites. In each plot, 40 plants consisting of ten clones (5 female and 5 male) were planted in five rows containing 8 plants each. During planting, minimum spacing between each plantlet and closest neighbouring plantlet was 20 cm. After planting, plantlets were watered in order to reduce drought stress, as well as reducing eventual differences in soil moisture between sites.

At each site, one HOBO Micro Station data-logger (Onset Computer Corporation, Cape Cod, Massachusetts, U.S.A.) was installed together with a PAR Smart Sensor (Onset Computer Corporation) and a Temperature/RH Smart Sensor (Onset Computer Corporation) (Fig. 1, Appendices Figs. A2 & A3 in Supplementary material).

In 2014, twelve 140 × 80 cm plots were established within each location. Prior to establishment, one large tree was removed from the location at 237 m a.s.l. with the purpose of equalising total irradiance received by plants across the different locations (Appendices Fig. A3 in Supplementary material). As the number of plants per plot was reduced to 20, these were evenly distributed between three rows with the same spacing between plants as the previous year. There were more males than females available for the experiment due to faster micropropagation of males, and thereby each plot had 8 females and 12 males. At each elevation, the twelve plots were arranged in a 4 × 3 matrix, yielding four blocks containing each of three treatment types: UV-B attenuation, covered control and uncovered control. Plots that were assigned to UV-B attenuation treatments were covered by Autostat CT5 polyester UV-B-attenuating filters (MacDermid Autotype Ltd., Wantage, UK) (see Appendices Fig. A1 in Supplementary material for transmittance spectrum), mounted on a 320 × 280 cm frame consisting of polypropylene (PP) tubes. Each frame was raised 1 m above the ground by four vertical PP-tubes, which were raised gradually as plants grew taller. As daily solar trajectories vary throughout the year, the frames were dimensioned and placed accordingly over the plots in order to provide attenuation of UV-B from direct solar radiation throughout the growing season. For this purpose, dimensions were based on recommendations on UV-B attenuation provided in Aphalo et al. (2012). Plots assigned to the controlled cover treatment had each a frame built in the same manner as for the UV-B attenuation treatment, but were covered by 50 μm translucent (82–90%) LD-polyethylene sheets instead of UV-B-filters. The purpose of this treatment was to separate the effect of UV-B attenuation from the effect of covering the plots with plastic sheets (Aphalo et al., 2012). The third treatment consisted of leaving the plots uncovered as a control for the effect of plastic sheet cover. Frames for covered treatment plots were installed on 7 July 2014 and kept until 1 October 2014, except for covered treatments at 237 m a.s.l. which were removed on 24 September 2014 due to damage from strong winds.

### 2.3. Plant measurements and recording of apical stages

Stem height and basal diameter of plants were measured after planting in the field and every third week throughout the growing season. Plant height was measured with a ruler as the distance from the tip of the highest stem to the ground surface. Basal diameter was

measured 1 cm from the soil surface using a digital calliper. In 2013, height was measured four times and diameter was measured three times, while in 2014, height was measured five times and diameter was measured four times. The three-stage system used for scoring apices during autumnal bud formation discerns between three stages: growing apex (1); green bud having closed bud scales (0.5); brown/red mature bud (0). In situations where green closed buds broke in autumn and apices resumed growth, apices were scored as growing (Strømme et al., 2015). Some plants were affected by *Venturia* shoot blight or grazed upon by intruding small herbivores. Excluding these, the number of measured and scored plants were 175 females and 205 males in 2013 and 246 females and 419 males in 2014. We also recorded bud break during spring 2014 and 2015. Bud break was dissected into four stages based on Fu et al. (2012), as reported in Strømme et al. (2015): closed bud (0); closed bud with visible green leaf tip (1); green leaf diverging from bud axis but no visible petiole (2); break bud with at least one visible petiole (3). During spring 2014, apices of all plants were recorded until all buds were fully broken. In spring 2015, apical scoring at 830 m a.s.l. was terminated before all plants had fully broken buds.

### 2.4. Sampling of plant material

In 2013, one plantlet from each clone was sampled from each plot at each elevation on 17 September, yielding a total of 115 harvested plants. In 2014, three female and three male plants were sampled from each of the three UV-B plot treatments at the lowermost elevation (237 m a.s.l.) on 17 September, 24 September and 7 October, yielding a total of 54 plants. Plants were sampled by cutting the stem 1 cm above the ground surface and removing the top 5 cm section of the stem. From the remaining stem, the top 10 cm section of each plantlet was cut and placed in a labelled paper bag containing silica gel, while the top- and lowermost leaf of the 10 cm stem section was placed in a separate labelled paper bag containing silica gel. The sampled plantlet parts were dried at 30 °C in a drying oven, grinded and kept in a freezer before analyses for C, N and phenolic compounds.

### 2.5. Chemical analyses

Grinded samples of leaves and stems were analysed for C and N concentrations using an Elementar Micro Cube (Elementar Analysen, Hanau, Germany). Concentrations of phenolic compounds was analysed using high performance liquid chromatography (HPLC)

Analyses of phenolic content followed earlier published methods (Nybakken et al., 2012). Of the powdered plant material, 10 mg were weighed and extracted separately using 600 µl of cold 100% methanol. The samples were homogenized for 30 s at 5500 rpm using a Precellys® 24 (Bertin Technologies, Île-de-France, France) and incubated in an ice bath for 15 min. The mixture was then centrifuged at 15,000 rpm for 3 min (Eppendorf® Centrifuge 5415R, Hamburg, Germany) and the supernatant collected. The residue was re-extracted twice with incubation in the ice bath. The three consecutive supernatants were pooled together, and the extract was evaporated to dryness using an Eppendorf® concentrator and stored in a freezer until further analysis. The salicylates, phenolic acids and flavonoids were analyzed by HPLC (Agilent, Series 1100, Germany), consisting of a binary pump (G1312A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A) and a diode array detector (G1315B). The phenolic metabolites were separated using a ODS Hypersil (4.6 × 60 mm) column (Thermo Fisher Scientific Inc, Waltham, USA). Prior to the HPLC analyses, the dried extract was resuspended in 400 µl methanol–water (50:50). A volume of 20 µl from each sample was injected and all runs were performed at +30◦C. The mobile phase consisted of two solvents: 0.25% o-phosphoric acid and 1.5% tetrahydrofuran in Milli-Q ultrapure water (Merck Millipore, Darmstadt, Germany), and methanol 100% with a flow rate of 2 ml $min^{-1}$ (Nybakken et al., 2012). The phenolic metabolites were identified by comparing their retention times and UV spectra with those of commercial standards. The following standards were used for quantification: salicin (Sigma-Aldrich Finland Oy, Helsinki, Finland) for salicin salicylates 1–10; salicortin (Sigma-Aldrich Finland Oy, Helsinki, Finland) for salicortin; cinnamic acid (Sigma-Aldrich Finland Oy, Helsinki, Finland) for *p*-OH-cinnamoyl salicortin and phenolic acids; tremulacin (Apin Chemicals, Abingdon, UK) for tremulacin; (+)-catechin (Fluka Chemie AG, Buchs, Switzerland) for (+)-catechin; quercetin 3-galactoside (Apin Chemicals, Abingdon, UK) for quercetin 3-arabinoglucoside and quercetin 3-glucuronide; myricetin 3-rhamnoside (Apin Chemicals, Abingdon, UK) for myricetin derivative; kaempferol 3-O-glucoside (Extrasynthese, Genay Cedex, France) for kaempferol 3-glucuronide; chlorogenic acid (Sigma-Aldrich Finland Oy, Helsinki, Finland) for chlorogenic acid and neochlorogenic acid.

### 2.6. Climate data

In both years, temperature, RH and irradiance was recorded on a ten-minute basis (Fig. 1), Appendices Figs. A2 & A3 in Supplementary material. There are two gaps in the data series from 2013, as some dataloggers malfunctioned before 28 August and after 22 September. In 2014, we recorded temperature and RH for three plots with different cover treatments. Recording occurred on a five-minute basis using EL-USB-1 temperature data loggers (Lascar Electronics, Salisbury Wiltshire, UK) (Appendices Fig. A4 in Supplementary material). Each logger was raised 10 cm above ground, and since three devices were used measurements were performed from 9 to 31 July at 237 m a.s.l, from 4 to 25 August at 575 m a.s.l, and from 27 August to 3 September at 830 m a.s.l. UV-B irradiance at the three different elevations (Appendices Fig. A3 in Supplementary material) was calculated using the Quick TUV Calculator provided by NCAR Earth System Laboratory (http://cprm.acd.ucar. edu/Models/TUV/Interactive_TUV/) with the following settings: latitude 61.47055, longitude 4.22208, 300 DU of ozone, ground albedo (reflectivity) 0.1 and cloudless sky, while remaining conditions were kept as calculator default.

### 2.7. Statistical analyses

We tested the effect of elevation (three levels), plantlet sex (two levels) and day of year on stem height, stem basal diameter, apical stages and C, N and phenolic compound concentrations of stems and leaves using the R software for statistical computing (R Core Team, 2015). Applications of statistical tests and model selection were based on procedures described in Zuur et al. (2009). Recorded temperature and irradiance at each site were significantly correlated with day of year, and were excluded from statistical models in order to avoid autocorrelation. In the analyses of data from the UV-B attenuation experiment in 2014, UV-B attenuation was included as a fixed factor (two levels) in addition to cover (two levels: with or without cover) in the models. This was done in order to test for possible effects of covering the plots with plastic sheets that are separate from UV-B attenuation. In cases where the cover factor proved to yield a significant effect it was kept in the model. In the model selection process, we included plant clone (random term) and plot (random term) by using the lmer function in the lme4 package (Bates et al., 2015) when their inclusion yielded improved models based on AIC comparison. In the opposite case, data were analysed using generalised least squares (gls) from the nlme package (Pinheiro et al., 2015) in R. If the effect of day of year was significant and nonlinear, we proceeded by analysing data using generalised additive models (gam) for gls models and generalised additive mixed models (gamm) for lmer models, using the gamm4 package (Wood and Scheipl, 2015) in R. Effects on apical stages were tested using cumulative link mixed models (clmm) in R by applying the clmm function in the Ordinal Package (Christensen, 2013). Cumulative link mixed models allow statistical tests where the response variable is categorical, and this method was deemed most suitable for analysis of

apical stages data as it allows for multiple categories.

## 3. Results

### 3.1. Climate data

Average temperatures measured between 28 August and 22 September were 11.1 ± 0.54 °C at 237 m a.s.l., 9.9 ± 0.52 °C at 575 m a.s.l. and 8.4 ± 0.53 °C at 830 m a.s.l. (Fig. 1). In 2014, the same period had a mean daily temperature of 11.4 ± 0.35 °C at 237 m a.s.l., 10.0 ± 0.39 °C at 575 m a.s.l. and 8.8 ± 0.39 °C at 830 m a.s.l (Fig. 1).

Daily irradiance measured in 2013 show that plants at 237 m a.s.l. received less sunlight than those at higher elevations (Appendices Fig. A3 in Supplementary material). Average daily irradiance was 274 ± 23.0 μmol $m^{-2} s^{-1}$ at 237 m a.s.l., 352 ± 29.2 μmol $m^{-2} s^{-1}$ at 575 m a.s.l. and 372 ± 33.7 μmol $m^{-2} s^{-1}$ at 830 m a.s.l. (Appendices Fig. A3 in Supplementary material). In early 2014, removal of some vegetation surrounding the site at 237 m a.s.l, resulted in increased irradiance levels there. For that year, average daily irradiance was 369 ± 22.1 μmol $m^{-2} s^{-1}$ at 237 m a.s.l., 413 ± 24.2 μmol $m^{-2} s^{-1}$ at 575 m a.s.l. and 370 ± 23.3 μmol $m^{-2} s^{-1}$ at 830 m a.s.l. (Appendices Fig. A3 in Supplementary material).

Measurements of relative humidity in 2013, recorded between 28 August and 22 September, were on average 86 ± 1.8% at 237 m a.s.l, 86 ± 1.9% at 575 m a.s.l and 87 ± 2.0% at 830 m a.s.l. (Appendices Fig. A2 in Supplementary material). Values recorded for 2014, between August 20 and September 27, were 86 ± 1.3% at 237 m a.s.l, 87 ± 1.1% at 575 m a.s.l and 88 ± 1.1% at 830 m a.s.l. (Appendices Fig. A2 in Supplementary material).

At 237 m a.s.l., average near-ground temperature and relative humidity measured between 9 and 31 July were 22.9 ± 0.58 °C and 67 ± 1.4% for UV-B attenuation, 22.8 ± 0.59 °C and 68 ± 1.3% for covered control and 22.1 ± 0.59 °C and 71 ± 1.6% for uncovered control, respectively (Appendices Fig. A4 in Supplementary material). At 575 m a.s.l., corresponding values measured between 4 and 25 August were 14.6 ± 0.79 °C and 82 ± 2.6% for UV-B attenuation, 15.0 ± 0.70 °C and 77 ± 1.8% for covered control and 15.0 ± 0.74 °C and 80 ± 2.2% for uncovered control, respectively (Appendices Fig. A4 in Supplementary material). At 830 m a.s.l., corresponding values measured between 27 August and 3 September were 11.9 ± 0.91 °C and 76 ± 2.4% for UV-B attenuation, 12.2 ± 0.97 °C and 75 ± 2.4% for covered control and 12.9 ± 1.16 °C and 75 ± 2.8% for uncovered control, respectively (Appendices Fig. A4 in Supplementary material).

### 3.2. Effects of elevation and UV-B attenuation on autumn and spring phenology

Autumnal bud formation occurred earlier at higher elevations (Table 1, Fig. 2) in 2013, as an average score of 0.5 was reached 35 days earlier for plant apices at 830 m a.s.l. compared to the average of plants at 237 m a.s.l. (Fig. 2). The significant interaction between elevation and plant sex in 2013 was due to male plants forming buds earlier than females at 237 m a.s.l., while the opposite was the case for plants grown at 575 ($P < 0.001$) and at 830 m a.s.l. ($P < 0.001$) (Fig. 2). All plants at 830 m a.s.l. and most plants at 575 m a.s.l. had completed bud formation by day 267 (24 September), while at 237 m a.s.l. there were 25 female and 35 male plants out of 91 and 96, respectively, which had not completed bud formation by that date (Fig. 2). These observations may be due to lower air temperature with increased elevation (Fig. 1). Earlier bud formation was also observed in 2014 at 830 m a.s.l., where apices of plants had an average score of 0.5 30 days earlier compared to 237 m a.s.l. However, this effect was overridden by UV-B attenuation ($P < 0.001$). This means that females and males under UV-B attenuation at 830 m a.s.l. had apical scores similar to plants at 237 m

**Table 1**
Results from statistical tests of measured responses of female and male (Male) *Populus tremula* grown at different elevations in 2013 and 2014 under different light treatments (UV-F) using the following functions in R: cumulative link mixed models (clmm), linear mixed effect models (lmer), generalised least squares (gls) models and generalised additive (gam) models. Results are given as *z*- (clmm) and *t*-values (gls, lmer, gam). For day of year (DOY) in gam models, results are given as *F*-values.

| | Test | UV-F x 830 m x Male | UV-F x 575 m x Male | UV-F x Male | UV-F x 830 m | UV-F x 575 m | UV-F | Cover | 830 m x Male | 575 m x Male | 830 m | 575 m | Male | DOY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2013 | | | | | | | | | | | | | | |
| Bud formation | clmm | | | | | | | | −6.49*** | −5.09*** | 20.02*** | 13.25*** | 0.91 | 29.44*** |
| Bud break | clmm | | | | | | | | | | −13.25*** | −4.93*** | | 29.89*** |
| Plant height | lmer | | | | | | | | 2.43*** | 2.92*** | −13.85*** | −11.83*** | −1.43 | 23.11*** |
| Plant basal diameter | lmer | | | | | | | | | | −8.52*** | −5.28*** | | 23.40*** |
| Leaf nitrogen | lmer | | | | | | | | | | −3.09** | −1.5 | | |
| Stem nitrogen | lmer | | | | | | | | 2.01* | 0.06 | 9.48*** | 5.90*** | 1.22 | |
| Leaf carbon | gls | | | | | | | | | | −1.96 | −2.30* | | |
| Stem carbon | gls | | | | | | | | | | | | −2.55* | |
| 2014 | | | | | | | | | | | | | | |
| Bud formation | clmm | | | | −3.61*** | −0.35 | 0.51 | | | | 5.65*** | 1.33 | | 34.58*** |
| Bud break | clmm | | | | | | | | | | −20.78*** | −8.88*** | | 37.73*** |
| Plant height | lmer | 4.10** | 1.19 | −2.53* | 0.03 | −0.6 | 0.03 | −2.68* | −3.10** | −1.09 | −5.33*** | −0.6 | 1.6 | 55.89*** |
| Plant basal diameter | lmer | 3.15** | 1.54 | −2.84** | −0.46 | −1.08 | 0.77 | −4.37** | −2.22* | −0.57 | −4.30*** | −0.37 | 2.03 | 41.41*** |
| Leaf nitrogen | gam | | | | | | −3.80*** | | | | | | 2.54* | 48.14*** |
| Stem nitrogen | gam | | | | | | −4.27*** | | | | | | −2.40* | 9.61*** |
| Leaf carbon | gam | | | | | | | | | | | | 5.73*** | 5.65*** |

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

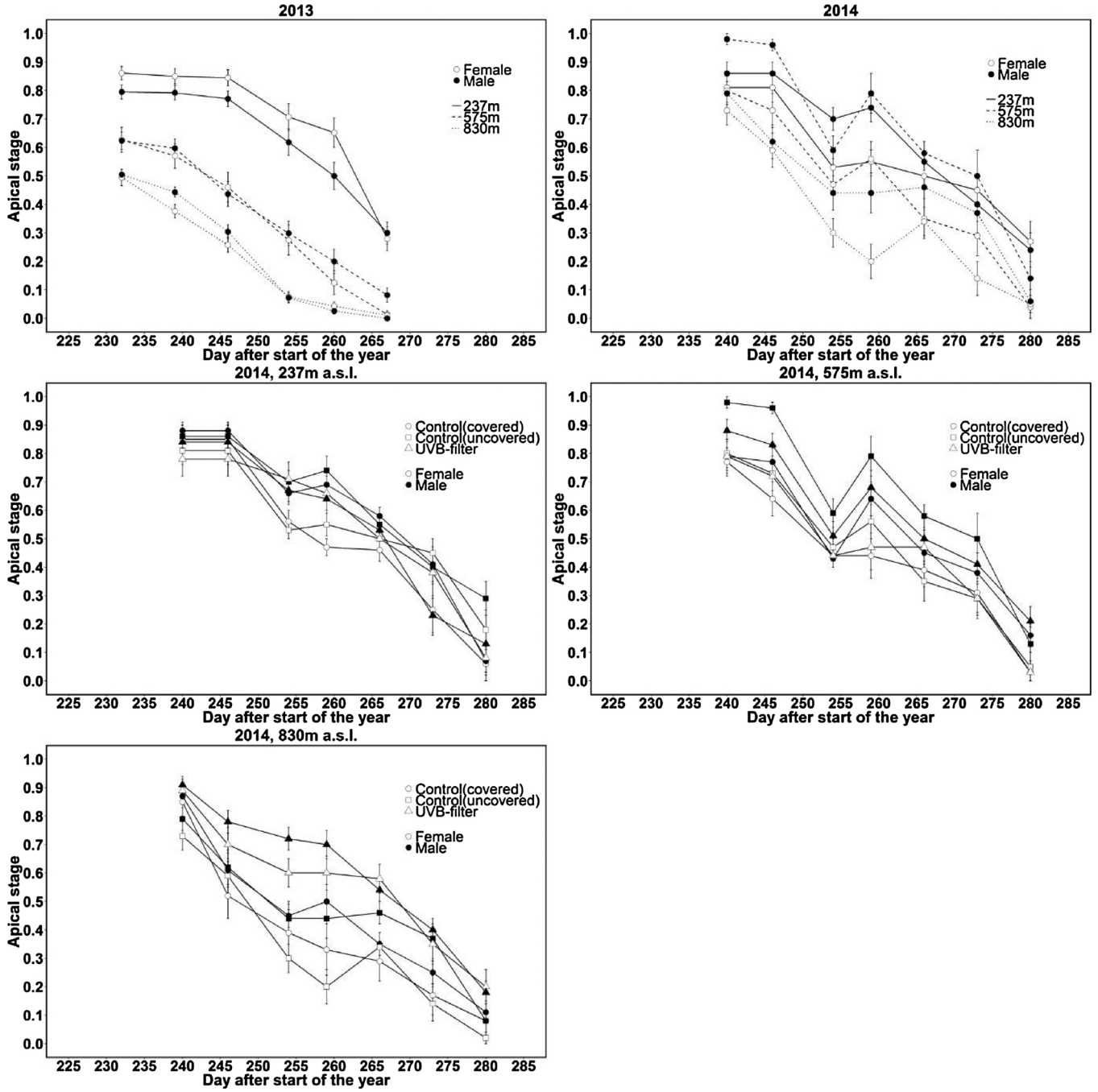


**Fig. 2.** Average apical scores ± 1SEM at different elevations during autumn in 2013 (175 females, 205 males) and 2014 (245 females, 419 males) (top panels), and at different elevations using UV-B attenuating and translucent cover treatments during autumn in 2014 (middle and bottom panels). Bud scores: growing apex (1); green bud having closed bud scales (0.5); brown/red mature bud (0).

a.s.l. throughout the growing season (Fig. 2). Higher UV-B levels at 830 m a.s.l. could explain why UV-B attenuation delayed bud formation only at this elevation, as calculated levels of UV-B irradiance increased slightly with elevation (Fig. 1). At 237 and 575 m a.s.l., average bud scores increased between day 254 (11 September) and 259 (16 September) in 2014 (Fig. 2) due to flushing of green closed buds and briefly resumed growth. In 2013 most plants at 575 and 830 m a.s.l. had completed bud formation around day 265 (22 September), while average scores for 2014 show that several plants had not completed bud formation on day 280 (7 October) (Fig. 2).

In spring 2014 and 2015, bud break occurred later at 575 ($P < 0.001$) and at 830 m a.s.l. ($P < 0.001$) compared to 237 m a.s.l. (Table 1, Fig. 3). For 2014, apices of plants growing at 830 m a.s.l. had an average score of 1.5 9 days later that those of plants at 237 m a.s.l., while in 2015 this difference was 23 days (Fig. 3). Neither plant sex, UV-B attenuation nor translucent cover treatments had any significant effects.

### 3.3. Effects of elevation and UV-B attenuation on plant size

Plants were smaller at higher elevations, both in terms of stem height and basal diameter in 2013 (Table 1, Figs. 4 and 5). By day 254 (11 September), plants at 237 m a.s.l. were 109 and 150% taller than plants at 575 and 830 m a.s.l., respectively, and corresponding values for basal stem diameter were 21 and 45%. Female plants were taller (19%) at 237 m a.s.l. compared to males in 2013, but these differences

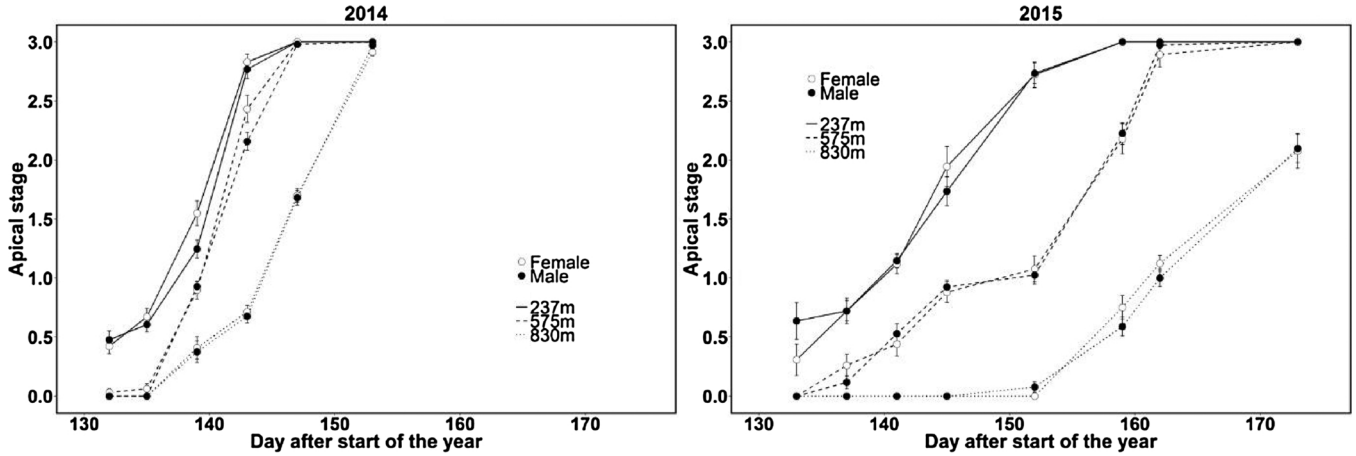


**Fig. 3.** Average apical scores ± 1SEM at different elevations during spring in 2014 (163 females, 188 males) and 2015 (150 females, 276 males, all treatments). Bud scores: closed bud (0); closed bud with visible green leaf tip (1); green leaf diverging from bud axis but no visible petiole (2); bud break with at least one visible petiole (3).

were less pronounced at 575 ($P = 0.003$) and 830 ($P = 0.015$) m a.s.l. given the significant interaction between sex and site (Table 1). Basal diameter was smaller at 575 (5%) ($P < 0.001$) and 830 m a.s.l. (29%) ($P < 0.001$) than at 237 m a.s.l. in 2013 (Fig. 5). In 2014, males were smaller than females in terms of height ($P = 0.001$) and basal diameter (P = 0.023) at 830 m a.s.l. However, UV-B attenuation yielded increased growth of male plants at this elevation both in terms of height ($P < 0.001$) and basal diameter ($P = 0.002$), given the significant interaction between UV-B attenuation and sex at 830 m a.s.l. (Table 1, Figs. 4 and 5). UV-B attenuation may have been the factor increasing stem height and basal diameter in males in 2014 by delaying bud formation at 830 m a.s.l. (Table 1; Figs. 4 and 5). For the same year, there was a negative effect of covering plots with either UV-B attenuation filters or translucent filter on stem height ($P = 0.012$) and basal diameter ($P < 0.001$) (Table 1). This effect yielded a 30 and 50% increase for uncovered plantlet height at 237 and 575 m a.s.l., respectively, while stem basal diameter decreased 21, 44 and 20% for uncovered plants at 237, 575 and 830 m a.s.l., respectively (Figs. 4 and 5).

### 3.4. Effects of elevation and UV-B attenuation on nutrient content

In 2013, leaves sampled on day 260 (17 September) at 237 m a.s.l. had 28% higher leaf N concentration than at 830 m a.s.l. ($P = 0.009$) (Tables 1 and 2). When compared to leaves sampled at 575 m a.s.l., N concentrations in leaves at 237 m a.s.l. were 11% higher, but this difference was non-significant (P = 0.160) (Tables 1 and 2). With increasing elevation, stem N concentrations were progressively higher, being 22% higher at 575 m a.s.l. ($P < 0.001$) and 70% higher at 830 m a.s.l. ($P < 0.001$) than the lowest elevation site (Tables 1 and 2). There was a significant interaction between elevation and plant sex at 830 m a.s.l. for stem N concentration, where it was higher for males than females ($P = 0.047$) (Tables 1 and 2). Leaf C concentrations differed only between plants grown at 575 and 237 m a.s.l., being lower in the former than the latter ($P = 0.01$) (Tables 1 and 2). For the whole experiment in 2013, female plants had higher stem C concentration (difference was only 1%) than males, irrespective of elevation ($P = 0.01$) (Tables 1 and 2).

In 2014, male plants at 237 m a.s.l. had higher (19%) leaf N concentration ($P = 0.014$) and lower (15%) stem N concentration ($P = 0.020$) than females (Table 1, Fig. 6). UV-B attenuation yielded lower N concentration in both stems ($P < 0.001$) and leaves ($P < 0.001$) for both sexes (Table 1, Fig. 6), as stems and leaves had 35 and 34% lower N concentration than control treatments, respectively. Males also had higher (3%) leaf C concentration ($P < 0.001$) (Table 1, Fig. 6), while none of the treatments yielded any significant effects on stem C.

### 3.5. Effects of elevation on phenolic content

Analyses of phenolic concentrations allowed determination of several groups of phenolic compounds in stems and leaves sampled in 2013 (Appendices Table A1 & A2 in Supplementary material) across elevations. For leaves sampled in 2013, salicylates constituted the largest proportion of the total concentration of phenolic compounds (73–76%), followed by flavonoids (15–18%) and phenolic acids (8–10%) (Appendices Table A1 in Supplementary material). Concentration of leaf salicylates was higher (18%) at 237 m a.s.l. compared to 575 m a.s.l. ($P < 0.001$), largely explaining why the concentration of combined leaf HPLC phenolics was higher (19%) at this elevation ($P < 0.001$) (Table 3). The total concentrations of leaf phenolic acids were lower at 575 (29%) ($P < 0.001$) and 830 m a.s.l. (23%) ($P < 0.001$) than at 237 m a.s.l. (Table 3). However, higher concentrations of phenolic acid 5 at 830 ($P = 0.043$) and phenolic acid 7 at 575 m a.s.l. ($P = 0.022$) were found in leaves of males, while phenolic acid 6 had a lower concentration for males at 830 m a.s.l. ($P = 0.012$) (Table 3).

Also for stems sampled in 2013, salicylates constituted the largest proportion of the concentration of combined HPLC phenolics (85–88%), followed by phenolic acids (5–8%) and flavonoids (0.5-1.5%) (Appendices Table A2 in Supplementary material). Higher concentrations of stem salicylates were measured at 575 (22%) ($P < 0.001$) and 830 m a.s.l. (31%) ($P < 0.001$) (Table 4) than at 237 m a.s.l. This was mainly due to increased concentrations of salicortin at 575 (25%, $P < 0.001$) and 830 (49%, $P < 0.001$) m a.s.l. (Table 4), which was the most abundant phenolic compound identified for stems for both years (Appendices Tables A2 & A6-A8 in Supplementary material). Higher concentrations of salicylate 6 were measured for males at 830 m a.s.l. ($P = 0.009$) (Table 4). Males at 830 m a.s.l. had reduced concentrations of phenolic acid 2, while males at 575 m a.s.l. had increased concentrations of phenolic acid 3 ($P = 0.045$) (Table 4).

### 3.6. Effects of UV-B on phenolic content

In leaves sampled at 237 m a.s.l. in 2014, salicylates constituted the largest proportion of the concentration of combined HPLC phenolics (65–84%), followed by flavonoids (10–26%) and phenolic acids (5–10%) (Appendices Tables A3-A5 in Supplementary material). UV-B attenuation yielded lower concentrations (39%) of leaf flavonoids ($P < 0.001$) (Table 5) due to the effects on concentrations of quercetin 3-glucuronide ($P < 0.001$) (Fig. 7), which had 55% lower concentrations under UV-B attenuation and was the most abundant flavonoid found in leaves for both years (Appendices Tables A1 & A3-A5 in

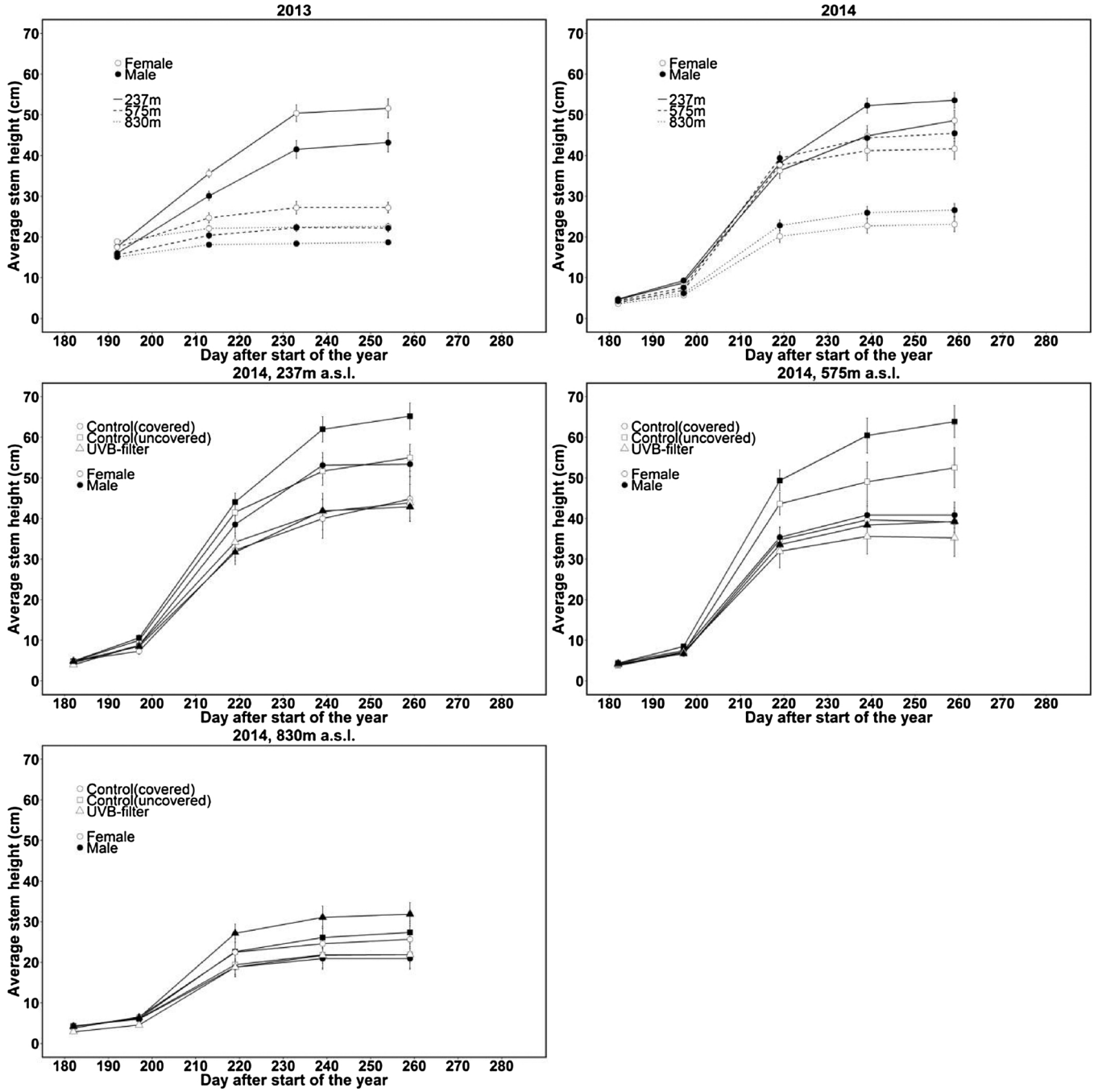


**Fig. 4.** Average stem height ± 1SEM of young *Populus tremula* plants at different elevations during autumn in 2013 (175 females, 205 males) and 2014 (245 females, 419 males) (top panels), and at different elevations using UV-B attenuating and translucent cover treatments during autumn in 2014 (middle and bottom panels).

Supplementary material). An opposite effect was found for salicin, which increased (22%) with UV-B attenuation ($P = 0.031$) (Table 5). Males had higher concentrations of phenolic acids (29%) and salicylates (26%) than females ($P < 0.001$), resulting in higher (22%) total HPLC phenolics ($P < 0.001$) (Table 5).

In stems sampled in 2014, salicylates constituted the largest proportion of total concentration of HPLC phenolics (81–92%), followed by phenolic acids (3–7%) and flavonoids (1.4-2.6%) (Appendices Table A6-A8 in Supplementary material). Salicylate concentration increased (17%) under UV-B attenuation ($P = 0.018$) and with sampling date ($P = 0.004$) (Fig. 7), yielding higher (14%) total HPLC phenolic content under UV-B attenuation ($P = 0.023$) (Table 6). Stems of females had higher concentrations of phenolic acids (21%, $P = 0.030$), flavonoids (61%, $P = 0.002$), as well as total concentration of phenolic compounds (14%, $P = 0.018$) (Table 6).

## 4. Discussion

Although global warming is expected to affect UV-B levels through impacts on the ozone layer, few studies have addressed the interactive effects of ambient temperature and UV-B on dioecious woody plants. Our data show that for females and males of *P. tremula*, variations of temperature and UV-B along a natural gradient may yield shifts within a single growing season in terms of growth and dormancy parameters, contents of C and N as well as concentrations of phenolic compounds. Along the elevational gradient, differences in growth properties between sexes reflected, and were likely affected by, the dissimilar responsiveness of autumn phenology to temperature.

Even though increasing elevation yielded both decreasing temperature and slightly increasing UV-B radiation (Fig. 1), results may

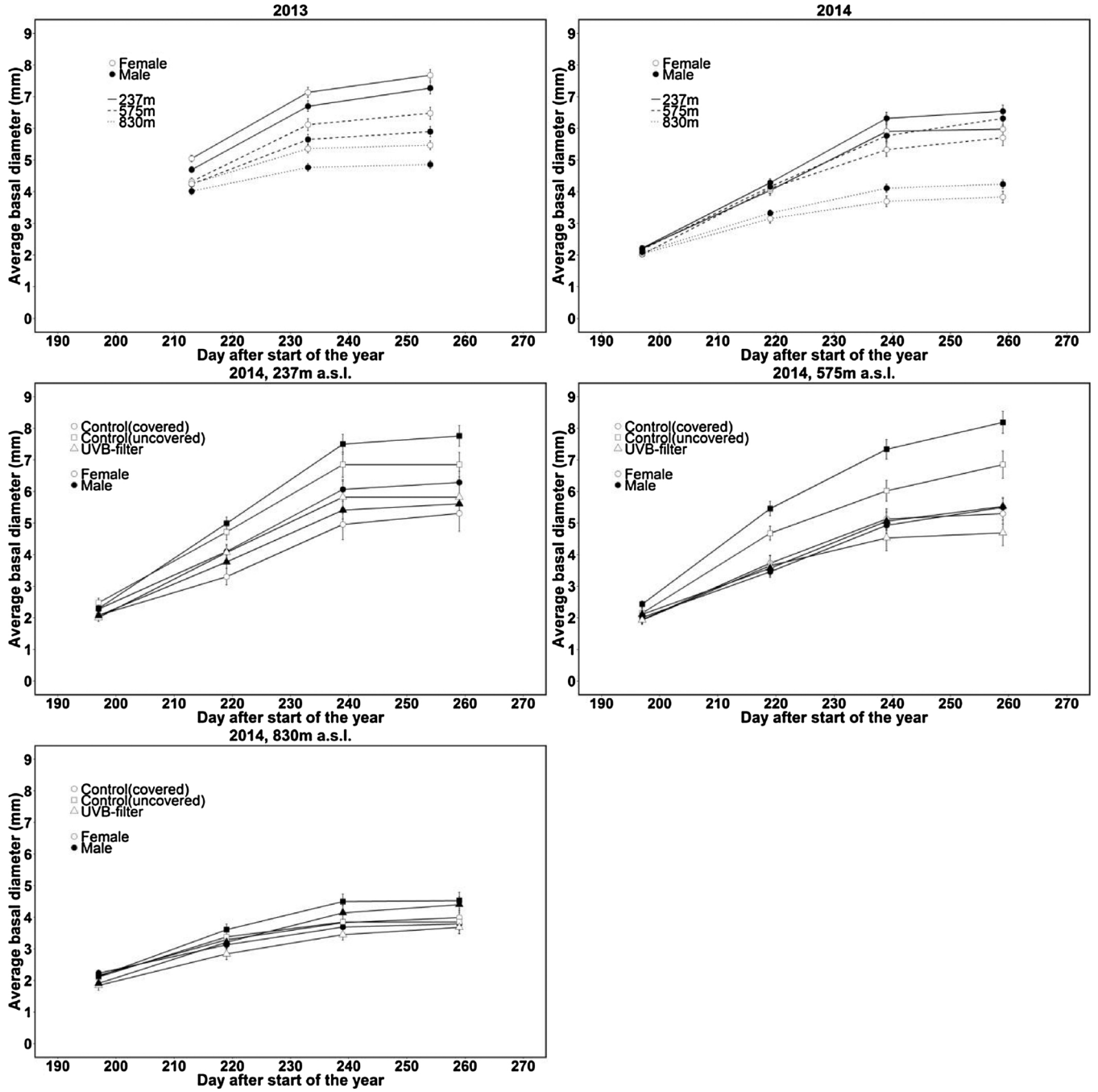


**Fig. 5.** Average stem basal diameter ± 1SEM of young *Populus tremula* plants at different elevations during autumn in 2013 (175 females, 205 males) and 2014 (245 females, 419 males) (top panels), and at different elevations and using UV-B attenuating and translucent cover treatments during autumn in 2014 (middle and bottom panels).

also have been affected by differences in irradiance between sites (Appendices Fig. A3 in Supplementary material), particularly in 2013 when the low-elevation site at 237 m a.s.l. received less solar radiation than the other sites. In this respect, prolonged growth in autumn at low elevation is likely due to higher temperature.

### 4.1. Effects of temperature and UV-B on phenology and plant size

In 2013, bud formation was delayed at low elevation, an effect which can be attributed to a longer growing season. This effect was significantly stronger in female plants ($P < 0.001$) (Table 1; Fig. 2). Evidence suggests these observations are mostly due to lower air temperature with increased elevation (Fig. 1), as high temperature has previously been reported to delay autumnal bud formation in field-grown *Populus* (Rohde et al., 2011a), with a stronger response for males in *P. tremula* (Strømme et al., 2015). Measured size properties (height and diameter) of plants decreased with elevation (Figs. 4 and 5), and the effect was also evident in the period when all plants had growing apices (Fig. 2). Thus, higher temperature promoted plant growth in terms of both promoting cell division and elongation in individual plants as well as extending the growing season.

Female plants were taller than males in 2013 (Fig. 4), but height differences between sexes were less pronounced with increased elevation (Table 1; Fig. 4). For young plants of *P. tremula*, warming has been shown to yield a stronger growth response in females than males (Randriamanana et al., 2015), and higher temperatures at low elevations may explain the observed height differences between females and males in 2013. In 2014, the application of UV-B attenuation filters

**Table 2**
C and N concentrations (%) of leaves and stems from females and males of *Populus tremula* sampled from different elevations on 17 September 2013.

| | Leaf | | Stem | |
|---|---|---|---|---|
| | C | N | C | N |
| 830 m | | | | |
| Females | 42.1 ± 0.31 | 2.07 ± 0.13 | 46.0 ± 0.35 | 2.0 ± 0.10 |
| Males | 43.5 ± 2.08 | 2.16 ± 0.21 | 45.9 ± 0.33 | 2.6 ± 0.09 |
| 575 m | | | | |
| Females | 42.8 ± 0.51 | 2.57 ± 0.08 | 46.6 ± 0.24 | 1.7 ± 0.08 |
| Males | 42.2 ± 0.40 | 2.30 ± 0.09 | 45.5 ± 0.21 | 2.0 ± 0.09 |
| 237 m | | | | |
| Females | 46.1 ± 1.02 | 2.70 ± 0.12 | 46.4 ± 0.29 | 1.2 ± 0.07 |
| Males | 43.8 ± 0.43 | 2.70 ± 0.11 | 45.7 ± 0.18 | 1.5 ± 0.09 |

increased stem and diameter growth for males at the highest elevation (Table 1; Figs. 4 and 5). This effect may be related to the observed male delay in bud formation under UV-B attenuation at this elevation (Table 1; Fig. 2), confirming that different combinations of temperature and UV-B yield dissimilar effects on growth. Another study involving *P. tremula* showed that enhanced UV-B levels in combination with warming yielded reduced stem height and basal diameter (Randriamanana et al., 2015), which is a similar effect as was suggested by our data.

Interestingly, male plants were bigger than female plants in 2014, both in terms of stem height and basal diameter (Figs. 4 and 5), which is opposite to results from 2013. This divergence may be related to slightly warmer and less fluctuating temperature in autumn 2014 (Fig. 1). Indeed, apical scores for autumn 2014 show that bud formation was delayed by approximately two weeks compared to the previous year (Fig. 2). Bud formation in *Populus* is known to occur in response to the sensing of a critical day-length (CDL), and warming has been shown to modify the sensitivity of this mechanism (Rohde et al., 2011a). Furthermore, high temperature has been found to delay the appearance of closed green buds more strongly in males than females of *P. tremula* (Strømme et al., 2017). The contrasting sex-related effects between 2013 and 2014 could thus be related to inter-annual variation in temperature before and after CDL-sensing, resulting in different warming effects on bud formation.

The observed delay in bud formation under UV-B attenuation at the highest elevation site is in line with findings from a previous field study, where a modulated increase of UV-B, using UV-B lamps in the field, promoted bud formation in males of young *P. tremula* plants (Strømme et al., 2015). The observed effects on bud phenology suggest that UV-B acts as an environmental signal affecting also autumn phenology in concert with temperature. Indeed, several studies show that UV-B acts as a regulatory signal for plant growth (Rozema et al., 1997; Jansen, 2002; Jenkins, 2009; Rizzini et al., 2011; Jansen and Bornman, 2012; Hayes et al., 2014; Roro et al., 2017). The observed delay in bud formation could be related to regulation of the growth hormone gibberellic acid (GA) by UV-B, as down-regulation of GA is known to yield apical bud formation in *Salix pentandra* and *P. tremula*. (Olsen et al., 1995a, 1995b, 1997a, 1997b; Mølmann et al., 2005). A recent study shows that UV-B detection in *A. thaliana* antagonises shade-avoidance responses mediated by auxin together with GA (Hayes et al., 2014). If GA levels in *P. tremula* are affected by UV-B through a similar signalling pathway, it is possible that UV-B attenuation in our study prevented UV-B from affecting GA levels. Increased levels of abscisic acid (ABA) in the apical

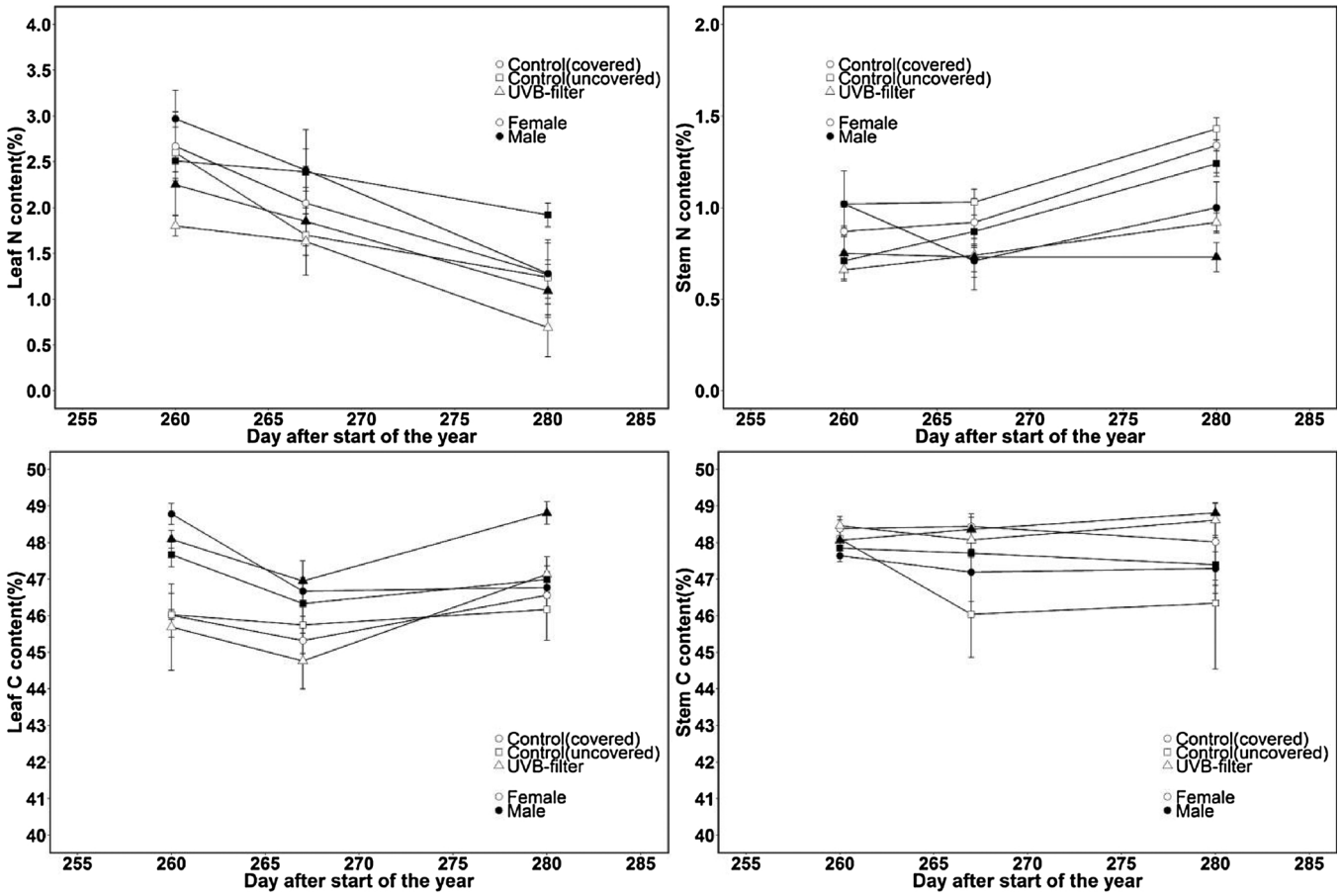


**Fig. 6.** Percent content of N (top panels) and C (bottom panels) in leaves (left panels) and stems (right panels) from females and males of *Populus tremula* under UV-B attenuating and translucent cover treatments at 237 m a.s.l. during autumn 2014.

**Table 3**
*t*-values for leaf concentrations of HPLC phenolics in females and males of *Populus tremula* grown at different elevations in 2013. *t*-values were obtained using linear mixed effect models (lmer) in R.

| | Test | 830 m x Male | 575 m x Male | 830 m | 575m | Male |
|---|---|---|---|---|---|---|
| Chlorogenic acid | lmer | | | −2.16* | −1.06 | |
| Neochlorogenic acid | lmer | | | −6.89*** | −6.22*** | |
| Phenolic acid 1 | lmer | | | −10.35*** | −10.20*** | |
| Phenolic acid 2 | lmer | | | −7.73*** | −7.77*** | |
| Phenolic acid 3 | lmer | | | −7.12*** | −5.37*** | |
| Phenolic acid 5 | lmer | 2.05* | 1.67 | 1.39 | −0.37 | −0.57 |
| Phenolic acid 6 | lmer | −2.56* | −0.45 | 5.50*** | 0.86 | 0.72 |
| Phenolic acid 7 | lmer | 0.55 | 2.33* | 0.69 | −0.78 | −0.61 |
| Quercetin arabinoglucoside | lmer | | | −0.61 | −2.42* | |
| Kaempferol 3-glucuronide | lmer | | | 4.29*** | 0.56 | |
| Salicylate 1 | lmer | | | 3.71** | 0.09 | |
| Tremulacin | lmer | | | 0.23 | −3.26** | |
| p-OH- cinnamoylsalicortin | lmer | | | −0.77 | −3.20** | |
| **Total leaf phenolic acids** | lmer | | | −3.63*** | −4.38*** | |
| **Total leaf salicylates** | lmer | | | −0.22 | −3.39*** | |
| **Total leaf HPLC phenolics** | lmer | | | −0.23 | −3.48** | |

Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

**Table 4**
*t*-values for stem concentrations of HPLC phenolics in females and males of *Populus tremula* grown at different elevations in 2013. *t*-values were obtained using the following statistical tests in R: generalised least squares model (gls), linear mixed effect model (lmer).

| | Test | 830 m x Male | 575 m x Male | 830 m | 575 m | Male |
|---|---|---|---|---|---|---|
| Chlorogenic acid derivative 1 | lmer | | | −2.65** | −1.08 | |
| Chlorogenic acid derivative 3 | lmer | | | 3.58*** | 2.38* | |
| Phenolic acid 2 | lmer | −2.03* | 0.80 | 4.00*** | 2.52* | −0.38 |
| Phenolic acid 3 | lmer | 0.53 | 2.54* | 1.64 | −0.49 | −1.92 |
| Phenolic acid 5 | lmer | | | | | 2.40* |
| Salicylate 4 | lmer | | | 3.04** | 3.91*** | |
| Salicylate 6 | lmer | 2.67** | 1.88 | −3.39*** | −1.66 | −2.36* |
| Salicylate 7 | lmer | | | −2.28* | −2.39* | |
| Salicylate 8 | lmer | | | | | −2.22* |
| Salicylate 9 | lmer | | | −2.67* | −1.94 | |
| Salicylate 10 | gls | | | | | 5.05*** |
| Salicin | lmer | | | 4.34*** | 3.51*** | |
| Salicortin | lmer | | | 6.64*** | 3.53*** | |
| Tremulacin | lmer | | | 0.21 | 2.24* | |
| Salireposide | lmer | | | 3.72*** | 3.05** | |
| **Total stem salicylates** | lmer | | | 5.44*** | 4.16*** | |
| **Total stem HPLC phenolics** | lmer | | | 4.78*** | 3.88*** | |

Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

**Table 5**
*t*- and *F*-values for leaf concentrations of HPLC phenolics measured on different dates (DOY) in females and males of *Populus tremula* grown in 2014 at 237 m a.s.l. under near-ambient UV-B levels (UV-B transmitting filters) UVB-attenuation filters (UV-F) and ambient UV-B levels (no cover). The effects of UVB-attenuation (UV-F) and covering plots (Cover) were tested separately. *t*- and *F*-values were obtained using the following statistical tests in R: generalised least squares model (gls), linear mixed effect model (lmer), generalised additive model (gam), generalised additive mixed effect model (gamm).

| | Test | UV-F | Cover | Male | DOY |
|---|---|---|---|---|---|
| Chlorogenic acid derivative | gls | | | | 3.89*** |
| Neochlorogenic acid | lmer | | −2.74** | | |
| Phenolic acid 1 | lmer | −3.08** | | | |
| Phenolic acid 2 | gls | | | 2.93** | |
| Phenolic acid 3 | gls | | −2.11* | 3.59*** | |
| Phenolic acid 4 | gls | | | | 2.75** |
| Phenolic acid 5 | lmer | | −2.32* | | |
| Phenolic acid 6 | gls | | | −2.94** | |
| Phenolic acid 7 | gamm | | | 2.41* | 7.25** |
| Kaempferol 3-glucuronide | lmer | | | | 2.79* |
| Quercetin arabinoglucoside isomer 1 | gls | | | | 2.98** |
| Quercetin arabinoglucoside isomer 2 | gam | | | 6.48*** | 11.47*** |
| Quercetin 3-glucuronide | gam | −4.34*** | | | 10.17*** |
| Salicin | gls | 2.22* | | | |
| Salicortin | gls | | | | 3.15** |
| **Total leaf phenolic acids** | gamm | | | 2.73** | 10.42*** |
| **Total leaf flavonoids** | gam | −3.67*** | | | 14.45*** |
| **Total leaf salicylates** | lmer | | | 3.77*** | |
| **Total leaf HPLC phenolics** | gam | | | 3.61*** | 6.72** |

Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

domain is also associated with autumnal bud formation in *Populus* during short days (Ruttink et al., 2007), and UV-B has been shown to increase ABA levels in leaves of *P. cathayana* (Xu et al., 2010). It is therefore also possible that bud formation in *P. tremula* is affected by UV-B through increased ABA levels.

Considering that bud formation occurred later in males at higher elevations in 2013, and that UV-B attenuation delayed bud formation in 2014, it is noteworthy that none of these effects were detected in the bud break data during spring 2014 and 2015. The delaying effect of increased elevation on bud break in both years can be related to thermal requirements, as spring warming has been shown to advance bud break in deciduous tree species (Fu et al., 2012). Also previous year autumn warming has been shown to yield earlier bud break in *P. tremula*, with a significantly stronger effect on male plants (Strømme et al., 2015). In this regards, earlier bud break at lower elevation could have been positively affected by higher autumn temperatures. Still, this effect cannot be disentangled from the effect of winter and spring temperatures across different elevations. One should also keep in mind that in this study, as in Strømme et al. (2015), we used juvenile plants growing closer to the ground surface than adult trees.

### 4.2. Effects of temperature and UV-B on plant nutrient content

Leaves and stems were sampled across the three different elevations at one event during autumn 2013, and analyses revealed that partitioning of N between leaves and stems differed with increased elevation (Table 1; Fig. 6). We measured increased stem N content with elevation, while leaf N content decreased, suggesting a higher N storage at high elevation. In *Populus*, N cycling and storage has been shown to be

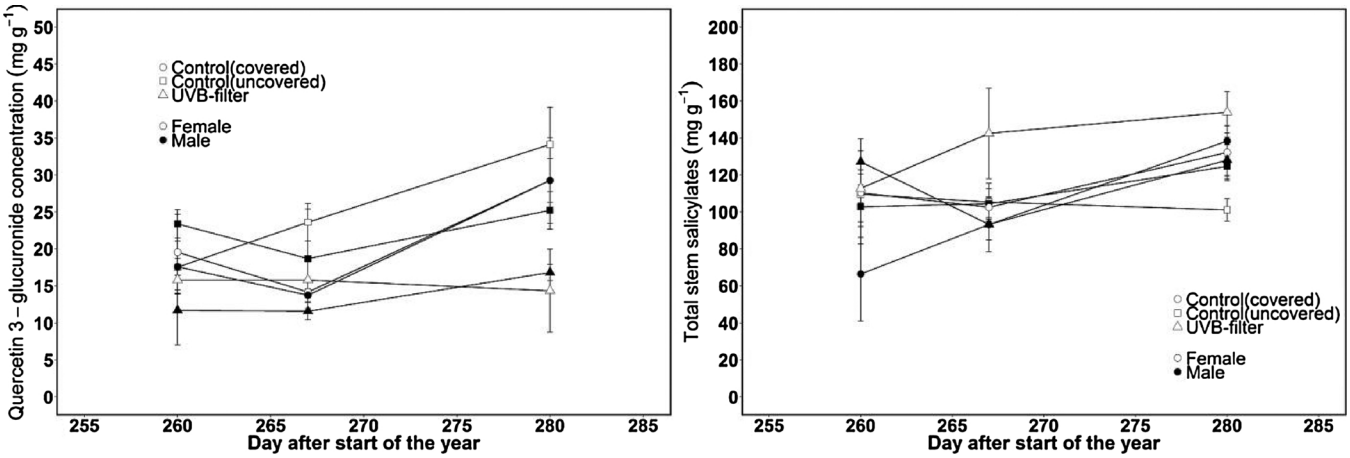


**Fig. 7.** Concentrations of quercetin 3-glucuronide in leaves (left) and total stem salicylates (right) for females and males of *Populus tremula* under UV-B attenuating and translucent cover treatments during autumn 2014.

**Table 6**
*t-* and *F-values* for stem concentrations of HPLC phenolics measured on different dates (DOY) in females and males of *Populus tremula* grown in 2014 at 237 m a.s.l. under near-ambient UV-B levels (UV-B transmitting filters) UVB-attenuation filters (UV-F) and ambient UV-B levels (no cover). The effects of UVB-attenuation (UV-F) and covering plots (Cover) were tested separately. *t-* and *F*-values were obtained using the following statistical tests in R: generalised least squares model (gls), linear mixed effect model (lmer), generalised additive model (gam).

| | Test | UV-F | Male | DOY |
|---|---|---|---|---|
| Phenolic acid 4 | gls | | | 2.04* |
| (+)-Catechin | gam | | −3.32** | 5.81* |
| Salicylate 7 | gls | | | 2.70** |
| Salicylate 8 | gls | | −2.43* | |
| Salicylate 9 | gls | | | 2.47* |
| Salireposide | gls | | −7.39*** | |
| **Total stem phenolic acids** | lmer | | −2.24* | |
| **Total stem flavonoids** | gam | | −3.32* | 5.81** |
| **Total stem salicylates** | gam | 2.47* | | 9.29** |
| **Total stem HPLC phenolics** | gam | 2.36* | −2.45* | 10.05** |

mediated by vegetative storage proteins (Cooke and Weih, 2005), and occurs in autumn with shorter days and lower temperatures (Thomas and Stoddart, 1980). As we sampled plant material across elevations only in one event, we are unable to account for the combined effects of day-length and temperature on N cycling in our study. However, warming has been reported to yield slightly higher N content in leaves of *P. tremula* (Randriamanana et al., 2015), which could explain the lower leaf N content found in our study at higher elevations where temperatures were lower. Across the three elevations, females had only 1% higher stem C concentrations compared to males. This may have been related to lower C assimilation in males, as the latter were smaller in terms of both height and basal diameter.

For 2014, when plants were sampled only at 237 m a.s.l. at three time points, the higher leaf N content and lower stem N content in males may be related to delayed N translocation with delayed phenology. The measured decrease of N content under UV-B attenuation is in line with findings from a previous UV-B enhancement study, where increased UV-B levels yielded higher N content in leaves of *P. cathayana* (Xu et al., 2010). However, increased UV-B has also been shown to decrease N content in leaves in rice (*Oryza sativa*) (Dai et al., 1992), and plant responses to UV-B may in this respect vary substantially between species (Zlatev et al., 2012).

### 4.3. Effects of temperature and UV-B on phenolic content

Analyses of phenolic concentrations of stems and leaves revealed that individual compounds and groups of compounds either increased or decreased with increased elevation (Tables 3 and 4, Appendices Tables A1 & A2 in Supplementary material). For leaf concentrations, elevation had a mainly negative effect on phenolic acids and salicortin (Table 3). In contrast, Bernal et al. (2013) found that phenolic acids in leaves of *Buxus sempervirens* increased with elevation, but this effect was only found for leaves sampled in June. As we found reduced concentrations with elevation in leaves sampled in September, it cannot be determined whether this contrasting result is due to seasonal variation or interspecific differences. Even so, enhanced temperature has been shown to decrease content of phenolic acids and salicortin in leaves of *S. myrsinifolia* (Veteli et al., 2002), while a recent study of *Vitis vinifera* shows that a 10/7 °C (day and night temperature, respectively) chilling treatment decreased concentrations of phenolic acids in leaves through cold stress (Król et al., 2015). It is possible that cold stress at high elevation reduced leaf phenolic acid concentrations in our study, considering that the daily average temperature at 830 m dropped to 4.5 °C on the sampling date (17 September, Day 260) (Fig. 1), with a minimum temperature of 2.9 °C (data not shown). Although elevation affected some individual compounds dissimilarly between sexes, this did not yield any differences in total leaf concentrations of phenolic groups. Interestingly, a positive effect of elevation was found for salicortin concentrations in stems (Table 4). This may be a result of concentrating effect, as plants were smaller in size with increased elevation.

Attenuation of UV-B in 2014 yielded lower concentrations of leaf flavonoids, mainly through lower concentrations of quercetin 3-glucuronide (Table 5, Appendices Tables 3–5 in Supplementary material), but in contrast to Feng et al. (2014), we did not find any sex-related differences in total flavonoid concentration under ambient UV-B. Different flavonoids provide protection against UV-B in higher plants (e.g. Li et al., 1993; Reuber et al., 1996), and enhanced UV-B has been shown to increase their concentrations in leaves of the woody boreal species *B. pendula* (Lavola et al., 1997), *S. myrsinifolia* (Tegelberg and Julkunen-Tiitto, 2001) and *P. tremula* (Lavola et al., 2013; Randriamanana et al., 2015). Phenolic acid 1 was similarly affected by UV-B attenuation, although this treatment did not affect the combined concentrations of phenolic acids (Table 5). Phenolic acids may have a role in protecting against UV-B (Sheahan, 1996; Lavola et al., 1997), and their concentrations have been shown also to increase with UV-B enhancement in *B. pendula* (Lavola et al., 1997). The combined concentration of leaf salicylates was not affected by UV-B attenuation, but the significant increase for salicin with this treatment is in line with a study involving *S. myrsinifolia* where enhanced levels of UV-B yielded lower concentrations of salicin (Tegelberg and Julkunen-Tiitto, 2001). Furthermore, the combined concentration of salicylates in stems increased under UV-B attenuation (Table 6, Fig. 7, Appendices Tables 6–8 in

Supplementary material), an effect that according to our knowledge has not been reported previously. Interestingly, males appeared to have better defended leaves against generalist herbivores than females due to the significantly higher concentrations of salicylates (Lindroth and Peterson, 1987; Ruuhola et al., 2001; Volf et al., 2015), while the opposite was the case for total stem salicylates.

In synthesis, we found that ambient levels of temperature and UV-B affect growth and defence of *P. tremula* in field conditions. These environmental factors are likely to shift during the next decades, and our data show that there are such responses to shifts within a single growing season in a widespread deciduous tree species. In addition, we observed sex-related responses to temperature and UV-B in terms of phenology, growth, and concentrations of assimilates and phenolics. However, we do not exclude that differences in irradiance and relative humidity also affected our results, as these climatic parameters varied slightly across elevations and light treatments. Still, plant growth was more pronounced at lower elevation for both years, which is likely a result of higher growth rates and delayed bud formation under warm temperatures. At the highest elevation, UV-B attenuation delayed bud formation and yielded increased male plant height and stem diameter. With increased elevation, concentration of N in leaves decreased while concentration of N in stems increased, suggesting that low temperature in autumn favoured translocation of N from leaves to stems. Both increased elevation and attenuation of UV-B yielded higher total phenolic concentrations in stems. This was mainly due to the resulting higher concentrations of salicylates, indicating possible trade-offs in accumulation between different phenolics. UV-B attenuation yielded lower leaf flavonoid concentration, but the combined concentrations of leaf phenolics remained unaffected. Interestingly, this treatment yielded increased concentration of stem salicylates together with the aforementioned size parameters, and thereby, contrary to our hypothesis, did not yield shifting allocation patterns from defence to growth. Whether UV-B has dissimilar effects on different phenolic synthesis pathways remains to be investigated further.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2017.09.013.

## References


Aphalo, P.J., Albert, A., McLeod, A., Heikkilä, A., Goméz, I., Figueroa, F.L., Robson, M.T., Strid, Å., 2012. Manipulating UV radiation. In: Aphalo, P.J., Albert, A., Björn, L.O., McLeod, A., Robson, M.T., Rosenqvist, E. (Eds.), Beyond the Visible: A Handbook of Best Practice in Plant UV Photobiology. Cost Action FA0906 UV4growth. University of Helsinki, Helsinki, pp. 35–70 pp.

Ballaré, C.L., Scopel, A.L., Stapleton, A.E., Yanovsky, M.J., 1996. Solar Ultraviolet-B radiation affects seedling emergence DNA integrity, plant morphology, growth rate, and attractiveness to herbivore insects in Datura ferox. Plant Physiol. 112, 161–170.

Ballaré, C.L., Rosseaux, M.C., Searles, P.S., Zaller, J.G., Giordano, C.V., Robson, T.M., Caldwell, M.M., Sala, O.E., Scopel, A.L., 2001. Impacts of solar ultraviolet-B radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina) An overview of recent progress. J. Photochem. Photobiol. 62, 67–77.

Ballaré, C.L., Caldwell, M.M., Flint, S.D., Robinson, S.A., Bornman, J.F., 2011. Effects of solar ultraviolet radiation on terrestrial ecosystems Patterns, mechanisms and interactions with climate change. Photochem. Photochem. Sci. 10, 226–241.

Bassman, J.H., Robberecht, R., 2001. Effects of enhanced UV-B radiation on growth and gas exchange in Populus deltoides Bartr. ex Marsh. Int. J. Plant Sci. 162, 103–110.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-Effects models using lme4. J. Statist. Software 67, 1–48. http://dx.doi.org/10.18637/jss.v067.i01.

Bernal, M., Llorens, L., Julkunen-Tiitto, R., Badosa, J., Verdaguer, D., 2013. Altitudinal and seasonal changes of phenolic compounds in Buxus sempervirens leaves and cuticles. Plant Physiol. Biochem. 70, 471–482.

Bertin, R.I., 2008. Plant phenology and distribution in relation to recent climate change. J. Torrey Bot. Soc. 135, 126–146.

Bornman, J.F., Barnes, P.W., Robinson, S.A., Ballaré, C.L., Flint, S.D., Caldwell, M.M., 2015. Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. Photochem. Photochem. Sci. 14, 88–107.

Caldwell, M.M., Ballaré, C.L., Bornman, J.F., Flint, S.D., Björn, L.O., Teramura, A.H., Kulandaivelu, G., Tevini, M., 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climate change factors. Photochem. Photobiol. Sci. 2, 29–38.

Christensen, R.H.B., 2013. Ordinal: ersion 2015. pp. 1–21.

Clapham, D., Dormling, I., Ekberg, I., Eriksson, G., Qamaruddin, M., Vince-Prue, D., 1998. Latitudinal cline of requirement for far-red light for the photoperiodic control of bud set and extension growth in *Picea abies* (Norway spruce). Physiol. Plant. 102, 71–78.

Cooke, J.E.C., Weih, M., 2005. Nitrogen storage and seasonal nitrogen cycling in *Populus*: bridging molecular physiology and ecophysiology. New Phytol. 167, 19–30.

Dai, Q., Coronel, V.P., Vergana, B.S., Barnesand, P.W., Quintos, A.T., 1992. 1; Ultraviolet-B radiation effects on growth and physiology of four rice cultivars. Crop Sci. 32, 1269–1274.

Fehér, B., Kozma-Bognár, L., É, Kevei, Hajdu, A., Binkert, M., Davis, S.J., Schäfer, E., Ulm, R., Nagy, F., 2011. Functional interaction of the circadian clock and UV RESISTANCE LOCUS 8-controlled UV-B signaling pathways in *Arabidopsis thaliana*. Plant J. 67, 37–48.

Feng, L., Jiang, H., Zhang, Y., Zhang, S., 2014. Sexual differences in defensive and protective mechanisms of *Populus cathayana* exposed to high UV-B radiation and low soil nutrient status. Physiol. Plant. 151, 434–445.

Fu, Y.H., Campioli, M., Deckmyn, G., Janssens, I.A., 2012. The impact of winter and spring temperatures on temperate tree budburst dates: results from an experimental climate manipulation. PLoS One 7, e47324.

Hänninen, H., Tanino, K.K., 2011. Tree seasonality in a warming climate. Trends Plant Sci. 16, 412–416.

Hartman, D.L., Klein Tank, A.M.G., Rusticucci, M., Alexander, L.V., Broenniman, B., et al., 2013. Observations: atmosphere and surface. In: Stocker, T.F., Qin, D. (Eds.), Climate Change 2013: The Physical Science Basis. Cambridge University Press, pp. 159–254.

Hayes, S., Velanis, C.N., Jenkins, G.I., Franklin, K.A., 2014. UV-B detected by the UVR8 photoreceptor. Proc. Natl. Acad. Sci. U. S. A. 111. http://dx.doi.org/10.1073/pnas.1403052111.

Hember, R.A., Kurz, W.A., Metsaranta, J.M., Black, T.A., Guy, R.D., et al., 2012. Accelerating regrowth of temperate-maritime forests due to environmental change. Global Change Biol. 18, 2026–2040.

Huiskes, A.H.L., Lud, D., Moerdijk-Poortvliet, T.C.W., 2001. Field research on the effects of UV-B filters on terrestrial Antarctic vegetation. Plant Ecol. 154, 77–86.

IPCC, 2014. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change. IPCC, Geneva (151 pp.).

Jacoby, G.C., D'Arrigo, R.D., 1995. ree ring width and density evidence of climatic and potential forest change in Alaska. Global Biogeochem. Cycles 9, 227–234.

Jansen, M.A.K., Bornman, J.F., 2012. UV-B radiation: from generic stressor to specific regulator. Physiol. Plant. 145, 501–504.

Jansen, M.A.K., 2002. Ultraviolet-B radiation effects on plants: induction of morphogenic responses. Physiol. Plant. 116, 423–429.

Jenkins, G.I., 2009. Signal transduction in responses to UV-B radiation. Annu. Rev. Plant Biol. 60, 407–431.

Jiang, H., Zhang, S., Feng, L., Korpelainen, H., Li, C., 2015. Transcriptional profiling in dioecious plant *Populus cathayana* reveals potential and sex-related molecular adaptations to solar UV-B radiation. Physiol. Plant. 153, 105–118.

Junttila, O., Kaurin, Å., 1985. Climatic control of apical growth cessation in latitudinal ecotypes of Salix pentandra L. In: Kaurin, Å., Junttila, O., Nilsen, J. (Eds.), Plant Production in the North. Norwegian University Press, Oslo pp 83–91.

Kalcsits, L., Silim, S., Tanino, K., 2009. Warm temperature accelerates short-photoperiod-induced growth cessation and dormancy induction in hybrid poplar (Populus x spp). Trees 23, 971–979.

Kauppi, P.E., Posch, M., Pirinen, P., 2014. Large impacts of climatic warming on growth of boreal forests since 1960. PLoS One 9, e111340.

Kotilainen, T., Venäläinen, T., Tegelberg, R., Lindfors, A., Julkunen-Tiitto, R., et al., 2009. Assessment of UV biological spectral weighting functions for phenolic metabolites and growth responses in silver birch seedlings. Photochem. Photobiol. 85, 1346–1355.

Koven, C.D., 2013. Boreal carbon loss due to poleward shift in low-carbon ecosystems. Nat. Geosci. 6, 452–456.

Król, A., Amarowicz, R., Weidner, S., 2015. The effects of cold stress on the phenolic compounds and antioxidant capacity of grapevine (*Vitis vinifera* L.) in leaves. J. Plant Physiol. 189, 97–104.

Kullman, L., 2001. 20th century climate warming and tree-limit rise in the southern Scandes of Sweden. Ambio 30, 72–80.

Lavola, A., Julkunen-Tiitto, R., Aphalo, P., de la Rosa, T., Lehto, T., 1997. The effect of u.v.-B radiation on u.v.-absorbing secondary metabolites in birch seedlings grown under simulated forest soil conditions. New Phytologist 137, 617–621.

Lavola, A., Nybakken, L., Rousi, M., Pusenius, J., Petrelius, M., Kellomäki, S., Julkunen-Tiitto, R., 2013. Combination treatment of elevated UV-B radiation, CO2 and temperature has little effect on silver birch (Betula pendula) growth and phytochemistry. Physiol. Plant. 149, 499–514.

Li, J., Ou-Lee, T.-M., Raba, R., Amundson, R.G., Last, R.L., 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5, 171–179.

Li, F.R., Peng, S.-L., Chen, B.-M., Hou, Y.-P., 2010. A meta-analysis of the responses of woody and herbaceous plants to elevated ultraviolet-B radiation. Acta Oecol. 36, 1–9.

Lindroth, R.L., Peterson, S.S., 1987. Effects of plant phenols of performance of southern armyworm larvae. Oecologia 75, 185–189.

Manney, G., Santee, M.L., Rex, M., Livesey, N.J., Pitts, M.C., Veefkind, P., Nash, R.R., Wohltmann, I., Lehmann, R., Froidevaux, L., Poole, L.R., Schoeberl, M.R., Haffner, D.P., Davies, J., Dorokhov, V., Gernandt, H., Johnson, B., Kivi, R., Kyro, E., Larsen, N., Levelt, P.F., Makshtas, A., McElroy, C.T., Nakajima, H., Parrondo, M.C., Tarasick, D.W., von der Gathen, P., Walker, P.K.A., Zinoviev, N.S., 2011. Unprecedented Arctic ozone loss in 2011, Arctic winter 2010/2011 at the brink of an ozone hole. Nature 478, 469–475.

McKenzie, R.L., Aucamp, P.J., Bais, A.F., Bjørn, L.O., Ilyas, M., Madronich, S., 2011. Ozone depletion and climate change: impacts on UV radiation. Photochem. Photobiol. Sci. 10, 182–198.
Menzel, A., Sparks, T., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K., Bissolli, P., Braslavská, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Å, Dahl, Defila, C., Donnelly, A., Filella, Y., Jatczak, K., Måge, F., Mestre, A., Ø, Nordli, Peñuelas, J., Pirinen, P., Remisová, V., Sheifinger, H., Stritz, M., Susnik, A., van Vliet, A.J.H., Wielgolaski, F.E., Zach, S., Zust, A., 2006. European phenological response to climate change matches the warming pattern. Global Change Biol. 12, 1969–1976.
Mølmann, J.A., Asante, D.K., Jensen, J.B., Krane, M.N., Ernstsen, A., Junttila, O., Olsen, J.E., 2005. Low night temperature and inhibition of GA biosynthesis overrides phytochrome action and induce bud set and cold acclimation, but not dormancy in *PHYA* overexpressors and wild-type of hybrid aspen. Plant Cell Environ. 28, 1579–1588.
Mølmann, J.A., Junttila, O., Johnsen Ø, Olsen, J.E., 2006. Effects of red, far-red and blue light in maintaining growth in latitudinal populations of Norway spruce (*Picea abies*). Plant Cell Environ. 29, 166–172.
Monleon, V.J., Lintz, H.E., 2015. Evidence of tree species' range shifts in a complex landscape. PLoS One 10, e0118069.
Morales, L.O., Tegelberg, R., Brosché, M., Keinänen, M., Lindfors, A., Aphalo, P.J., 2010. Effects of solar UV-A and UV-B radiation on gene expression and phenolic accumulation in Betula pendula leaves. Tree Physiol. 30, 923–934.
Nitsch, J.P., 1957. Growth responses of woody plants to photoperiodic stimuli. Proce. Am. Soc. Hortic. Sci. 70, 512–525.
Nybakken, L., Hörkkä, R., Julkunen-Tiitto, R., 2012. Combined enhancements of temperature and UVB influence growth and phenolics in clones of the sexually dimorphic Salix myrsinifolia. Physiol. Plant. 145, 551–564.
Nybakken, L., Julkunen-Tiitto, R., 2013. Gender differences in *Salix myrsinifolia* at the pre-reproductive stage are little affected by simulated climate change. Physiol. Plant. 147, 465–476.
Olsen, J.E., Lee, Y.K., 2011. Trees and boreal forests. In: Storey, K.B., Tanino, K.K. (Eds.), Temperature Adaptation in a Changing Climate: Nature at Risk. CAB International, Wallingford pp. 160–178.
Olsen, J.E., Jensen, E., Junttila, O., Moritz, T., 1995a. Photoperiodic control of endogenous gibberellins in seedlings of *Salix pentandra*. Physiol. Plant. 93, 639–644.
Olsen, J.E., Moritz, T., Jensen, E., Junttila, O., 1995b. Lack of effect of photoperiod on metabolism of exogenous GA19 and GA1 in *Salix pentandra* seedlings. Physiol. Plant. 94, 522–528.
Olsen, J.E., Junttila, O., Nilsen, J., Eriksson, M.E., Martinussen, I., Olsson, O., Sandberg, G., Moritz, T., 1997a. Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. Plant J. 12, 1339–1350.
Olsen, J.E., Junttila, O., Moritz, T., 1997b. Long day-induced bud break in *Salix pentandra* is associated with transiently elevated levels of GA1 and gradual increase in IAA. Plant Cell Physiol. 38, 536–540.
Olsen, J.E., Lee, Y.K., Junttila, O., 2014. Effect of alternating day and night temperature on short day-induced bud set and subsequent bud burst in long days in Norway spruce. Front. Plant Sci. 5. http://dx.doi.org/10.3389/fpls.2014.00691.
Opseth, L., Holefors, A., Rosnes, A.K.R., Lee, Y.K., Olsen, J.E., 2016. *FTL2* expression preceding bud set corresponds with timing of bud set in Norway spruce under different light quality treatments. Environ. Exp. Bot. 121, 121–131.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R., 2015. Nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3. pp. 1–122.
R Core Team, 2015. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Randriamanana, T.R., Lavola, A., Julkunen-Tiitto, R., 2015. Interactive effects of supplemental UV-B and temperature in European aspen seedlings: implications for growth, leaft traits, phenolic defence and associated organisms. Plant Physiol. Biochem. 93, 84–93. http://dx.doi.org/10.1016/j.plaphy.2015.03.001.
Ren, J., Dai, W., Xuan, Z., Yao, Y., Korpelainen, H., Li, C., 2007. The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. For. Ecol. Manage. 239, 112–119.
Ren, J., Duan, B., Zhang, X., Korpelainen, H., Li, C., 2010. Differences in growth and physiological traits of two poplars originating from different altitudes as affected by UV-B radiation and nutrient availability. Physiol. Plant. 138, 278–288.
Reuber, S., Bornman, J.F., Weissenböck, G., 1996. A flavonoid mutant of barley (Hordeum vulgare L.) exhibits increased sensitivity to UV radiation in the primary leaf. Plant Cell Environ. 19, 593–601.
Rizzini, L., Favory, J.J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schäfer, E., Nagy, F., Jenkins, G.I., Ulm, R., 2011. Perception of UV-B by the *Arabidopsis* UVR-8 protein. Science 332, 103–106.
Roberts, A.M.I., Tansey, C., Smithers, R.J., Phillimore, A.B., 2017. Predicting a change in the order of spring phenology in temperate forests. Global Change Biol. 21, 2603–2611.
Rohde, A., Bastien, C., Boerjan, W., 2011a. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. Tree Physiol. 31, 472–482.
Roro, A.G., Dukker, S.A.F., Melby, T.I., Solhaug, K.A., Torre, S., Olsen, J.E., 2017. UV-B-induced inhibition of stem elongation and leaf expansion in pea depends on modulation of gibberellin metabolism and intact gibberellin signalling. J. Plant Growth Regul. 36, 680–690.
Rozema, J., van de Staaij, J., Björn, L.O., Caldwell, M., 1997. UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. 12, 22–28.
Ruttink, T., Arend, M., Morreel, K., Storme, V., Rombauts, S., Fromm, J., Bhalerao, R.P., Boerjan, W., Rohde, A., 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19, 2370–2390.
Ruuhola, T., Tikkanen, O.P., Tahvanainen, J., 2001. Differences in host use efficiency of larvae of a generalist moth, Operophtera brumata on three chemically divergent Salix species. J. Chem. Ecol. 27, 1595–1615.
Schaphoff, S., Reyer, C.P.O., Schepaschenko, D., Gerten, D., Shvidenko, A., 2016. Tamm Review: Observed and projected climate change impacts on Russia's forests and its carbon balance. For. Ecol. Manage. 361, 432–444.
Serreze, M.C., Walsh, J.E., Chapin III, F.S., Osterkamp, T., Dyurgerov, M., Romanovsky, V., Oechel, W.C., Morison, J., Zhang, T., Barry, R.G., 2000. Observational evidence of recent change in the northern high-latitude environment. Clim. Change 46, 159–207.
Sheahan, J.J., 1996. Sinapate esters provide greater UV-B attenuation than flavonoids in *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. 83, 679–686.
Singh, J., Singh, R.P., 2014. Adverse effects of UV-B radiation on plants growing at Schirmacher Oasis, East Antarctica. Toxicol. Int. 21, 101–106.
Snyder, P.K., Delire, C., Foley, J.A., 2004. Evaluating the influence of different vegetation biomes on the global climate. Clim. Dyn. 23, 279–302.
Søgaard, G., Johnsen Ø, Nilsen, J., Junttila, O., 2008. Climatic control of bud burst in nine young seedlings of nine provenances of Norway spruce. Tree Physiol. 28, 311–320.
Strømme, C.B., Julkunen-Tiitto, R., Krishna, U., Lavola, A., Olsen, J.E., Nybakken, L., 2015. UV-B and temperature enhancement affect spring and autumn phenology in Populus tremula. Plant Cell Environ. 38, 867–877.
Strømme, C.B., Julkunen-Tiitto, R., Olsen, J.E., Nybakken, L., 2017. High daytime temperature delays autumnal bud formation in Populus tremula under field conditions. Tree Physiol. 37, 71–81.
Tanino, K., Kalcsits, L., Silim, S., Kendall, E., Gray, G.R., 2010. Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant Mol. Biol. 73, 49–65.
Tegelberg, R., Julkunen-Tiitto, R., 2001. Quantitative changes in secondary metabolites of dark-leaved willow (*Salix myrsinifolia*) exposed to enhanced ultraviolet-B radiation. Physiol. Plant. 113, 541–547.
Tegelberg, R., Julkunen-Tiitto, R., Aphalo, P.J., 2001. The effects of long-term elevated UV-B on the growth and phenolics of field-grown silver birch (*Betula pendula*). Global Change Biol. 7, 839–848.
Tegelberg, R., Veteli, T., Aphalo, P.J., Julkunen-Tiitto, R., 2003. Clonal differences in growth and phenolics of willows exposed to elevated ultraviolet-B radiation. Basic and Applied. Ecology 4, 219–228.
Terfa, M.T., Roro, A.G., Olsen, J.E., Torre, S., 2014. Effects of UV radiation on growth and postharvest characteristics of three pot rose cultivars grown at different altitudes. Sci. Hort. 178, 184–191.
Thomas, H., Stoddart, J.L., 1980. Leaf senescence. Annu. Rev. Plant Physiol. 31, 83–111.
Tognetti, R., 2012. Adaptation to climate change of dioecious plants: does gender balance matter? Tree Physiol. 32, 1321–1324.
Tsegay, B.A., Lund, L., Nilsen, J., Olsen, J.E., Mølmann, J.A., Ernsten, A., Junttila, O., 2005. rowth responses of *Betula pendula* ecotypes to red and far-red light. Electronic. J. Mol. Biol. 8, 17–23.
Veteli, T.O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H., Tahvanainen, J., 2002. Effects of elevated $CO_2$ and temperature on plant growth and herbivore defensive chemistry. Global Change Biol. 8, 1240–1252.
Volf, M., Hrcek, J., Julkunen-Tiitto, R., Novotny, V., 2015. To each its own; differential response of specialist and generalist herbivores to plant defense in willows. J. Anim. Ecol. 84, 1123–1132. http://dx.doi.org/10.1111/1365-2656.12349.
Wareing, P.F., 1956. Photoperiodism in woody plants. Annu. Rev. Plant Physiol. 7, 191–214.
Weiser, C.J., 1970. Cold resistance and injury in woody plants. Science 169, 1269–1278.
Williamson, C.E., Zepp, R.G., Lucas, R.M., Madronich, S., Austin, A.T., Ballaré, C.L., Norval, M., Sulzberger, B., Bais, A.F., McKenzie, R.L., Robinson, S.A., Häder, D.P., Paul, N.D., Bornman, J.F., 2014. Solar ultraviolet radiation in a changing climate. Nat. Clim. Change 4, 434–441.
Wood, S., Scheipl, F., 2015. Gamm4: Generalized Additive Mixed Models Using Mgcv and Lme4. R Package Version 2015. pp. 2–3.
Xu, X., Yang, F., Xiao, X., Zhang, S., Korpelainen, H., Li, C., 2008. Sex-specific responses of *Populus cathayana* to drought and elevated temperatures. Plant Cell Environ. 31, 850–860.
Xu, X., Zhao, H., Zhang, X., Hänninen, H., Korpelainen, H., Li, C., 2010. Different growth sensitivity to enhanced UV-B radiation between male and female Populus cathayana. Tree Physiol. 30, 1489–1498.
Zhang, Y., Feng, L., Jiang, H., Zhang, Y., Zhang, S., 2017. Different proteome profiles between male and female *Populus cathayana* exposed to UV-B radiation. Front. Plant Sci. 8. http://dx.doi.org/10.3389/fpls.2017.00320.
Zhao, H., Li, Y., Duan, B., Korpelainen, H., Li, C., 2009. Sex-related adaptive responses of *Populus cathayana* to photoperiod transitions. Plant Cell Environ. 32, 1401–14011.
Zhao, H., Li, Y., Zhang, X., Korpelainen, H., Li, C., 2012. Sex-related and stage-dependent source-to-sink transition in *Populus cathayana* grown at elevated $CO_2$ and elevated temperature. Tree Physiol. 32, 1325–1338.
Zhu, K., Woodall, C.W., Clark, J.S., 2012. Failure to migrate: lack of tree range expansion in response to climate change. Climate Change Biol. 18, 1042–1052.
Zlatev, Z.S., Lidon, F.J.C., Kaimakanova, M., 2012. Plant physiological responses to UV-B radiation. Emirates J. Food Agric. 24, 481–501.
Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer Verlag, Berlin, pp. 101–142.